VOLUME 35 DECEMBER 1957 NUMBER 6

Canadian Journal of Zoology

Editor: T. W. M. CAMERON

Associate Editors:

N. J. BERRILL, *McGill University*
I. McT. COWAN, *University of British Columbia*
E. M. DuPORTE, *Macdonald College, McGill University*
F. E. J. FRY, *University of Toronto*
F. R. HAYES, *Dalhousie University*
D. S. RAWSON, *University of Saskatchewan*
W. E. RICKER, *Biological Station, Nanaimo*
J. L. TREMBLAY, *Laval University*
V. B. WIGGLESWORTH, *Cambridge University*

Published by THE NATIONAL RESEARCH COUNCIL
OTTAWA CANADA

CANADIAN JOURNAL OF ZOOLOGY

(Formerly Section D, Canadian Journal of Research)

Under the authority of the Chairman of the Committee of the Privy Council on Scientific and Industrial Research, the National Research Council issues THE CANADIAN JOURNAL OF ZOOLOGY and five other journals devoted to the publication, in English or French, of the results of original scientific research. Matters of general policy concerning these journals are the responsibility of a joint Editorial Board consisting of: members representing the National Research Council of Canada; the Editors of the Journals; and members representing the Royal Society of Canada and four other scientific societies.

EDITORIAL BOARD

Representatives of the National Research Council

R. B. MILLER, *University of Alberta*
H. G. THODE, *McMaster University*
D. L. THOMSON, *McGill University*
W. H. WATSON (Chairman), *University of Toronto*

Editors of the Journals

D. L. BAILEY, *University of Toronto*
T. W. M. CAMERON, *Macdonald College*
H. E. DUCKWORTH, *McMaster University*
K. A. C. ELLIOTT, *Montreal Neurological Institute*
LÉO MARION, *National Research Council*
R. G. E. MURRAY, *University of Western Ontario*

Representatives of Societies

D. L. BAILEY, *University of Toronto*
Royal Society of Canada
T. W. M. CAMERON, *Macdonald College*
Royal Society of Canada
H. E. DUCKWORTH, *McMaster University*
Royal Society of Canada
Canadian Association of Physicists
K. A. C. ELLIOTT, *Montreal Neurological Institute*
Canadian Physiological Society
R. G. E. MURRAY, *University of Western Ontario*
Canadian Society of Microbiologists
H. G. THODE, *McMaster University*
Chemical Institute of Canada
T. THORVALDSON, *University of Saskatchewan*
Royal Society of Canada

Ex officio

LÉO MARION (Editor-in-Chief), *National Research Council*
F. T. ROSSER, Vice-President (Administration and Awards), *National Research Council*

Manuscripts for publication should be submitted to Dr. Léo Marion, Editor-in-Chief, Canadian Journal of Zoology, National Research Council, Ottawa 2, Canada.
(For instructions on preparation of copy, see **Notes to Contributors** (inside back cover))

Proof, correspondence concerning proof, and orders for reprints should be sent to the Manager, Editorial Office (Research Journals), Division of Administration and Awards, National Research Council, Ottawa 2, Canada.

Subscriptions, renewals, requests for single or back numbers, and all remittances should be sent to Division of Administration and Awards, National Research Council, Ottawa 2, Canada. Remittances should be made payable to the Receiver General of Canada, credit National Research Council.

The journals published, frequency of publication, and prices are:

Canadian Journal of Biochemistry and Physiology	Monthly	$3.00 a year
Canadian Journal of Botany	Bimonthly	$4.00
Canadian Journal of Chemistry	Monthly	$5.00
Canadian Journal of Microbiology	Bimonthly	$3.00
Canadian Journal of Physics	Monthly	$4.00
Canadian Journal of Zoology	Bimonthly	$3.00

The price of single numbers of all journals is 75 cents.

Canadian Journal of Zoology

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOLUME 35 DECEMBER 1957 NUMBER 6

SPHAERULARIA UNGULACAUDA SP. NOV. (NEMATODA: ALLANTONEMATIDAE) FROM THE DOUGLAS FIR BEETLE, DENDROCTONUS PSEUDOTSUGAE HOPK., WITH KEY TO SPHAERULARIA SPECIES (EMENDED)[1]

M. A. KHAN[2]

Abstract

A new species of nematode, *Sphaerularia ungulacauda* (Allantonematidae), is described from the Douglas fir beetle, *Dendroctonus pseudotsugae* Hopk., from British Columbia, Canada. The diagnostic characters of the genus *Sphaerularia* are emended and a key to species is given.

Introduction

In 1837, Dufour (3) established the genus *Sphaerularia* with *S. bombi* from bumblebee queens as the type species. Two species were added to this genus recently (6, 7), and in this paper one more is described. All three of these recent species were collected from bark beetles of the genus *Dendroctonus*.

Allantonematidae Chitwood and Chitwood, 1937

SPHAERULARIINAE FILIPJEV, 1929

Sphaerularia ***ungulacauda*** sp. nov. (Figs. 1–5)

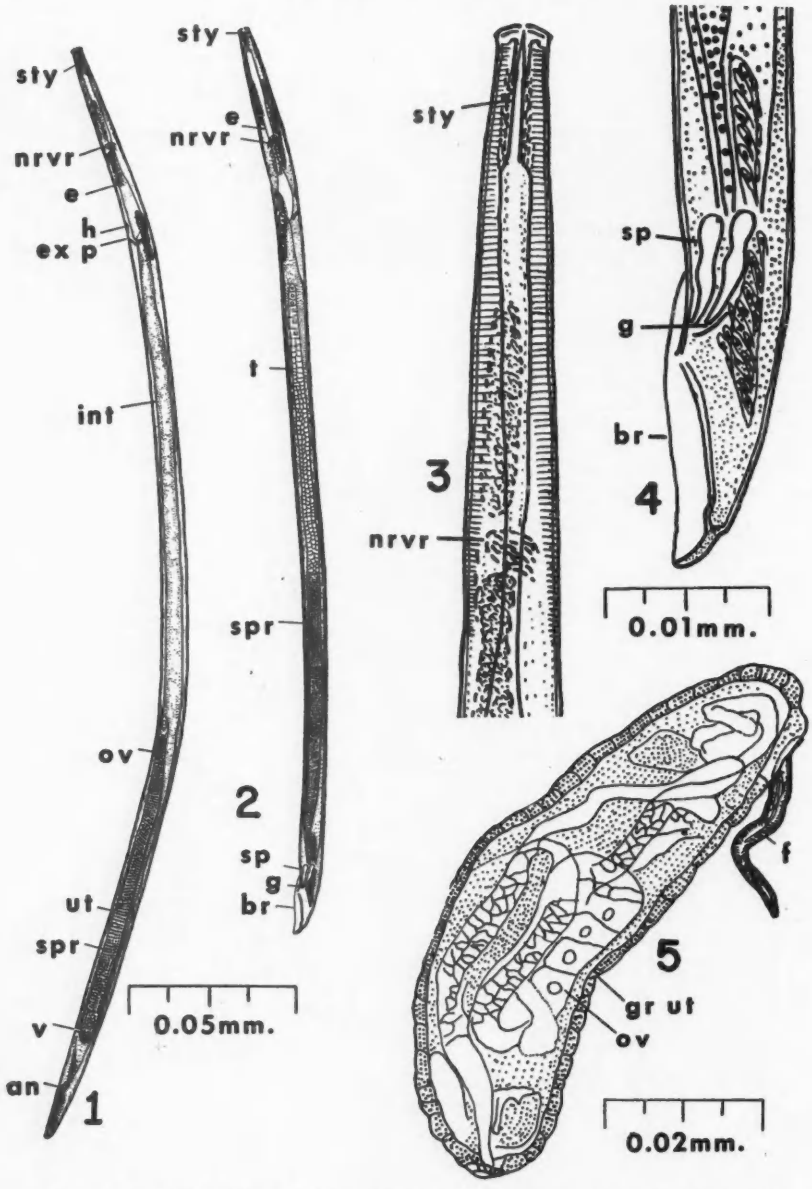
Female

Before eversion of the uterus, length 0.4 mm.; $a = 40$, $b = 6.3$, $c = 21$, and $V = 91\%$. After eversion, gravid uterus 0.84 mm. long and 0.24 mm. wide.

Body cylindrical, slightly tapering towards both ends and with well-marked, fine annulations in the cervical region. Lips fused and lip region rounded, cap-shaped, and set off by a slight constriction. Spear jointed, with small basal knobs, and 11–13 μ long. Esophagus consisting of a slender precorpus and a slightly enlarged corpus followed by a narrow isthmus leading into a well-developed basal bulb without a valve. Nerve ring crossing the base of the corpus. Hemizonid present. Excretory pore just behind the hemizonid. Esophagointestinal valve not seen. Vulva with slightly raised border; vagina a short tube with minute convex folds leading into a well-

[1] Manuscript received July 23, 1957.
Contribution No. 3671, Entomology Division, Science Service, Department of Agriculture, Ottawa, Canada.
[2] Nematode Section, Entomology Laboratory, Ottawa.

[The previous number of Can. J. Zoology (*35*, 581–633) was issued October 23, 1957.]



FIGS. 1–5. *Sphaerularia ungulacauda* sp. nov. 1. Female. 2. Male. 3. Head, female. 4. Tail. 5. Female with evaginated uterus.

ABBREVIATIONS: *an*, anus; *br*, bursa; *e*, esophagus; *ex p*, excretory pore; *f*, female; *g*, gubernaculum; *gr ut*, gravid uterus; *h*, hemizonid; *int*, intestine; *nrv r*, nerve ring; *ov*, ovary; *sp*, spicule; *spr*, spermatozoa; *sty*, stylet; *t*, testis; *ut*, uterus; *v*, vulva.

developed uterus extending anteriorly. A rudimentary postvulval uterine sac present. The uterus packed with spermatozoa, giving it a granular appearance. Ovary short, reflexed at the tip. The tail tapering gradually to a rounded end about one-fourth as wide as the body width.

The everted and hypertrophied uterus has widely rounded ends. The wrinkled body of the female is attached at one end and there is a minute opening at the other. The uterine sac contains convoluted ovarian tubes.

Male

Length 0.5 mm.; $a = 36$, $b = 5$, $c = 19.6$. The body markings and general appearance like those of the female before prolapse of the uterus. Testis outstretched or reflexed at the tip, about two-thirds as long as the entire worm. Spicules wavy; gubernaculum slightly curved when viewed from the side. Caudal bursa present and covering the entire tail and anteriorly the heads of the spicules. The tail short and tapering to a long, clawlike process.

Larvae

Only preadult stages were seen in both sexes. The female larva showing the outline of the uterus and the positions of the vulva and vagina. Testis visible in the male larva, but no evidence of spicules, gubernaculum, or caudal bursa. Tail of male larva shorter and thicker than that of the female larva and without the claw-shaped terminal process characteristic of the adult male.

Diagnosis

The features that differentiate *S. ungulacauda* from other species of *Sphaerularia* are: (*a*) the clawlike terminus of the male tail, (*b*) forward extension of the caudal bursa anteriorly, to the level of the heads of the spicules, (*c*) the granular appearance of the long, thin-walled uterus, and (*d*) the knobbed appearance of the head.

Hosts

Adults of the Douglas fir beetle, *Dendroctonus pseudotsugae* Hopk.

Type locality

Vernon, British Columbia, Canada.

Type specimen

Collection No. 591 of the Canadian National Collection of Nematodes, Ottawa, Ontario.

Bionomics

The adult females and preadult larvae were seen free inside the abdominal cavity of the host. The males were not seen inside the beetles, but were collected from strips of blotting paper used for packing the beetles in transit. As the male larvae seen inside the host were in the preadult stage, it seems likely that they leave the host just before molting into adults. Adult females were also collected from the packing material and showed

sperms in their uteri, which were not everted. Similar females were collected from the abdominal cavities of the beetles. These observations suggest that mating takes place outside the host body and that the fertilized female works its way back into the host for further development.

Systematics

Dufour (3) set up the genus *Sphaerularia* for the nematode *S. bombi* collected from bumblebee queens, *Bombus terrestris* L. and *B. hortorium* L. Filipjev (4) erected the subfamily Sphaerulariinae for nematodes living in the body cavity of insects. Filipjev and Schuurmans Stekhoven, Jr. (5), placed in this subfamily 11 genera of nematodes in which parasitic females grow into a reproductive sac with a barely distinguishable alimentary system, namely, *Sphaerularia*, *Attractonema*, *Bradynema*, *Allantonema*, *Howardula*, *Aphelenchulus*, *Parasitylenchus*, *Tylenchinema*, *Scatonema*, *Heterotylenchus*, and *Chondronema*. Later Chitwood (2), on the basis of prolapse of the uterus as a common character, retained only the genera *Sphaerularia* and *Tripius* in this subfamily and discarded the name *Attractonema*, a homonym, in favor of *Tripius* (1).

Genus *Sphaerularia* Dufour, 1837

With the addition of three new species to the genus *Sphaerularia* a revision of its description seems necessary, as some characters of the new forms are not covered by the previous description. Males in all species except *S. bombi* have a caudal bursa. *S. bombi* and the other species differ in the size of the evaginated uterus. In *S. bombi* it is 6–10 mm. long compared with 1–2 mm. in the other species. It is not proposed at this stage to divide this genus into two subgenera or genera, but further study of the group may reveal other forms and features so that some change may become desirable. An emended description of the genus *Sphaerularia* and key to the species follows.

Diagnosis emended. Parasites of insects. Lips amalgamated. Lip region flat or slightly rounded and cap-shaped. Cuticle with well-defined annulations in the cervical region. Spear knobbed, jointed, and well developed. Only anterior part of the spear visible in female with evaginated uterus. Esophageal musculature weak, basal bulb slightly overlapping the intestine. Vulva posterior, ovary single. Male also parasitic in some species, and with a caudal bursa in all species except *S. bombi*. Spicules and gubernaculum present. Testis single and outstretched or reflexed.

Type species: S. bombi.

KEY TO SPECIES OF *Sphaerularia*

(Males)

1.	Male found in the host body	4
	Male not found in the host body	2
2.	Male with a caudal bursa	3
	Male without a caudal bursa	*bombi* Dufour
3.	Tip of the male tail pointed like a claw	*ungulacauda* sp. nov.
4.	Tip of the male tail rounded, with subterminal papillae	*hastata* Khan
	Tip of the male tail rounded, without subterminal papillae	*dendroctoni* Massey

(Females with and without evaginated uteri)

1. Evaginated uterus 5–10 mm. long, sausage-shaped, and with wartlike surface elevations. Parasitic in bumblebee queens..... *bombi* Dufour
   Evaginated uterus 0.8–1.6 mm. long, saccate, and without wartlike surface elevations. Parasitic in bark beetles..... 2
2. Before evagination, uterus lined with polyhedral cells and without sperms..... 3
   Before evagination, uterus not lined with polyhedral cells and full of sperms ..... *ungulacauda* sp. nov.
3. Anterior half of the stylet slender. Parasitic in the Engelmann spruce beetle..... ..... *dendroctoni* Massey
   Anterior half of the stylet like an inverted 'V'. Parasitic in Douglas fir and mountain pine beetles..... *hastata* Khan

Discussion

Among the entomogenous nematodes the genus *Sphaerularia* is noteworthy because of peculiar characteristics of the female worm, of which the fertilized reproductive organs are completely everted. The gravid uterus expands greatly and the rest of the worm shrinks until it appears like a wrinkled appendage to the hypertrophied uterus. The nematode, therefore, not only draws heavily for nourishment on the host insect but also interferes with the growth and functions of the organs in its abdominal cavity.

The nematodes in the genus *Sphaerularia* play an important economic role. In some areas *S. bombi* is a menace to the cultivation of red clover and alfalfa. Its presence in bumblebee queens retards the growth of their ovaries so that they can neither lay eggs nor start new colonies (8). This results in reduced populations of bumblebees, which are required for the pollination of these important forage crops. However, the observations on the bionomics of the other three species suggest that these may be beneficial to mankind as they are parasitic on bark beetles, which are serious pests of forests and destroy large quantities of lumber every year. Massey (7) observed a sharp fall in egg production in the Engelmann spruce beetle infested with *S. dendroctoni*, the female of which closely resembles *S. hastata* and *S. ungulacauda* in parasitic behavior.

Acknowledgment

Thanks are due to Dr. A. D. Baker, Officer-in-Charge, Nematode Section, Ottawa, for his helpful suggestions in this work.

References

1. Chitwood, B. G. Nomenclatorial notes. I. Proc. Helminthol. Soc. Wash. D.C. **2**, 51–54 (1935).
2. Chitwood, B. G. and Chitwood, M. B. An introduction to nematology. Section 1. Monumental Printing Company, Baltimore, Md. 1950.
3. Dufour, L. Recherches sur quelques entozoires et larves parasites des insects orthopteres et hymenopteres. Ann. sci. nat. Zool. **1**, 5–20 (1837).
4. Filipjev, I. N. Classification of free living nematodes and relations to parasitic forms. J. Parasitol. **15**, 281–293 (1929).
5. Filipjev, I. N. and Schuurmans Stekhoven, Jr. A manual of agricultural helminthology. E. J. Brill, Leiden, Holland. 1941.
6. Khan, M. A. *Sphaerularia bombi* Duf. (Nematoda: Allantonematidae) infesting bumblebees and *Sphaerularia hastata* sp. nov. infesting bark beetles in Canada. Can. J. Zool. **35**, 519–523 (1957).
7. Massey, C. L. Nematode parasites and associates of the Engelmann spruce beetle (*Dendroctonus engelmanni* Hopk.). Proc. Helminthol. Soc. Wash. D.C. **23**, 14–24 (1956).
8. Palm, N. Normal and pathological history of the ovaries in *Bombus* Latr. (Hymenopt.) Opuscula Entomol. Suppl. **7**, 1948.

LIFE HISTORY, NON-SPECIFICITY, AND REVISION OF THE GENUS CHORIOPTES, A PARASITIC MITE OF HERBIVORES[1]

GORDON K. SWEATMAN[2]

Abstract

Chorioptic mange mites have been reared *in vitro* on epidermal debris. The life history of the mite has been observed, and each stage in the cycle described. Mites from the cow, horse, goat, sheep, and llama have been shown to be identical biologically and morphologically, and the specific names of *equi*, *caprae*, and *ovis* have been synonymized with *C. bovis*. The *in vitro* life cycle has been completed on epidermal debris from a variety of wild Cervidae, Bovidae, and Equidae, as well as on material from several breeds of domestic cattle, horses, sheep, and goats. From these and other data, three additional species or subspecies of *Chorioptes* were synonymized also with *C. bovis*. Only one other species, namely *C. texanus*, remains in the genus.

Introduction

Mange on domestic herbivores has been recognized since antiquity. Centuries passed, however, before an animalcule was associated with the disease, and according to Gerlach (7), it was Kegelaar in 1835 who first associated a dermatitis on cattle with what is known now as *Chorioptes bovis* (Héring, 1845) Gervais and Van Beneden, 1859. The mites and the disease have been recognized as being seasonal, appearing mainly in winter when cattle are indoors, but essentially disappearing in summer when the animals are on pasture. Even in winter, chorioptic mange is generally mild and restricted to small patches on the hind quarters. It only infrequently irritates the cow to an extent that interferes with milk or meat production. There is little doubt that the dermatitis is in some way associated with the mites, but according to the findings of Sweatman (26), there is no direct relationship between the lesions and *C. bovis*. Sweatman (26) demonstrated also that *C. bovis* has a predilection for the feet of domestic cattle, and that a large and general infestation of *C. bovis*, followed by the manifestation of lesions, could be induced by simply stanchioning cattle indoors in summer as well as during the previous winter.

Although chorioptic mange is of importance in world agriculture, not much is known about even the structure of *C. bovis*. In 1857, Gerlach (7) stated that *C. bovis* was morphologically identical (he examined only the adult stages) with *C. equi* from the horse, but maintained that each was host specific. *C. equi* has received a little more attention than *C. bovis*, but the only reasonably adequate description of any species in the genus is that of *C. caprae*

[1]Manuscript received June 28, 1957.
Contribution from Animal Pathology, Health of Animals Division, Canada Department of Agriculture, in co-operation with the Institute of Parasitology, Macdonald College P.O., Que., Canada.
[2]Animal Pathology, Health of Animals Division, Canada Department of Agriculture, Montreal Area Branch Laboratory, Macdonald College.

from a goat made by Oudemans in 1926 (19). In the present study, therefore, there is included a morphological description of the various stages in the life cycle of *C. bovis*.

Virtually nothing has been written on the life history of any of the eight species or subspecies in the genus. Two main technical obstacles have confronted researchers. Firstly, the annual summer reduction in the number of mites that made mites essentially unavailable at that season; and secondly, the minute size of the mite compared with its host, indicating the need for a suitable technique to rear or even maintain one or few mites at a time for study. As indicated previously (26), the first obstacle has been overcome by simply keeping cattle continuously indoors. This facilitated a build-up of the mite population to phenomenal numbers, making mites available at all times. The problem of the contrasting size of the parasite and its host was conquered by designing a suitable *in vitro* technique for rearing *C. bovis*. These methods made it possible to study the biology of the organism. The technique was used also for other species of *Chorioptes*, and experimental studies are presented on the comparative biology and morphology of the different species or subspecies in the genus.

Materials and Methods

Animals were examined for natural infestations by scraping epidermic debris and hair from a prescribed site on an animal and examining the material and its container for mites under a stereoscopic microscope. Mites preserved for further study were either fixed in 70% alcohol with added glycerine, or were mounted directly in Hoyer's Medium. Morphological studies were carried out under the oil immersion of a Zeiss phase-contrast microscope. Measurements were made with an ocular micrometer, and the bodies of the mounted specimens, when measured, were slightly enlarged by compression of the cover glass.

In vitro Culture Method

All previous attempts to rear any of the mange mites off the host have failed. In the current study, a technique has been developed for rearing reproducing populations of *C. bovis* for periods of 3 to 8 months, although it seemed impossible to maintain them indefinitely. The best source for beginning cultures was a foot scraping of a cow, or for that matter, from almost any part of an animal with a heavy general infestation. Mites, together with epidermic debris and hair in the scraping, were placed in a series of vials that, in turn, were maintained in a jar with a capacity of about a gallon (Fig. 1). The lid of the jar was large enough to permit freedom of the hand and wrist within it. Paraffin in the bottom of the jar was at least a centimeter deep, and contained appropriate excavations for holding about 10 vials. A solution of sulphuric acid on the paraffin maintained the relative humidity at 80% according to the data of Buxton (3). The relative humidity was

checked from determination of the specific gravity of the solution, and by use of a dew-point apparatus. It was necessary to circumvallate the lip of each vial with lead in order to keep the vial upright in the paraffin when the solution was present. The solution also served as a moat preventing the exchange of mites between vials. Vials were of a height (5 cm.) that permitted them to be used directly under a stereoscopic microscope (Spencer). Both paraffin and lead were suitable to use with sulphuric acid, since the former, and the product of the latter (lead sulphate), are inert. Bolting silk, held in place with elastic bands, was used to retain mites within the vials, but even with it, it was impossible to prevent loss of some individuals. This problem, however, was aided somewhat by the mites themselves. After being in the vials 3 or 4 days, many mites displayed a kind of "homing instinct" and apparently became content with their artificial environment. It was then possible to remove the bolting silk without fear of losing the mites. The jar was sealed with petroleum jelly and placed in an incubator at 35° C. This simple technique was suitable for rearing mites through sequential generations for some months. By transferring mites to vials with unused epidermic debris it was possible to perpetuate them longer. It was possible also to start a culture with a single individual, for example a bred ovigerous female, and anticipate an increase in the number of individuals in subsequent generations.

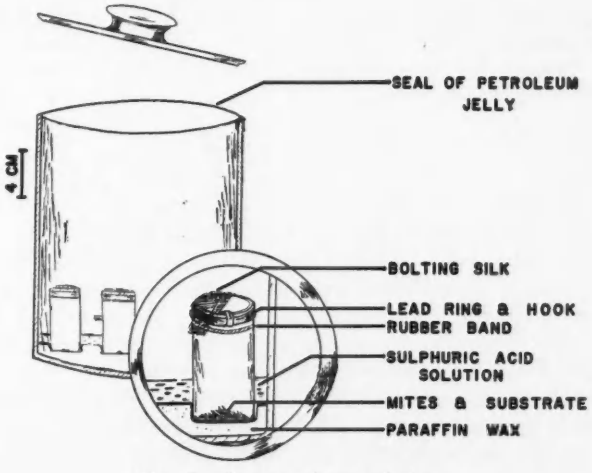


FIG. 1. *In vitro* culture technique.

The maintenance of the mites under high humidity and temperature encouraged the growth of remarkably few fungi, but fungi did sometimes occur and not infrequently used dead mites as focal points of growth. Even when present, they appeared of little significance in the survival of mites in the cultures.

I. Morphology and Life History

A. Description of Stages

Chorioptes bovis is an obligatory, non-burrowing mite that passes all stages on the host. In a single cycle, the mite passes through five stages—the egg, larva, protonymph, deutonymph, and adult. These terms will be used for the sequential development of the male, but in keeping with the terminology used by previous authors the female deutonymph and adult female will be called the pubescent female and ovigerous female respectively.

Larva (Figs. 3–13, 15–16, 18, 22–24)

(Measurements in microns with the mean in parentheses and the standard deviation of individuals (measured 15) in the second set of parentheses. The parts referred to in the text are labelled in Fig. 2.)

An elongate oval mite with distinct shoulders and rather short legs. It is soft-bodied. No sexual dimorphism was noted. Translucent white immediately after hatching, but soon becomes a brownish-gray color.

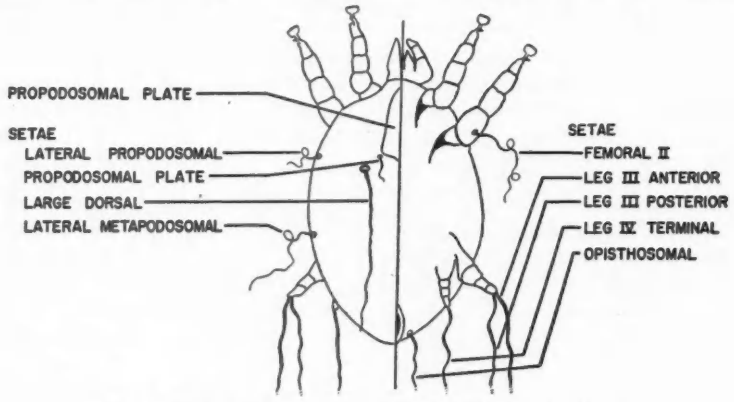


Fig. 2. Structural nomenclature used for chorioptic mites.

Dorsum

Length 131–236 (191) (24) and width 82–175 (146) (21).

Sclerotized propodosomal plate in the general shape of an isosceles triangle, but variable in detail (Figs. 4 to 11), with a pair of setae near, or sometimes attached to the posterior corners. Plate elevated medially (Fig. 5). Remainder of integument finely striated. One pair of quite long dorsal setae arise posterolaterally to the plate and measure 87–112 (105) (7). Two pairs of dorsolateral setae occur, the propodosomal being 20–38 (34) (3) and the metapodosomal 58–81 (71) (9). Also, five other pairs of short idiosomal setae are present. These infrequently vary in relative position as in Figs. 22 to 24. The pair that is marginal, however, is invariably more terminal than the corresponding pair on subsequent stages.

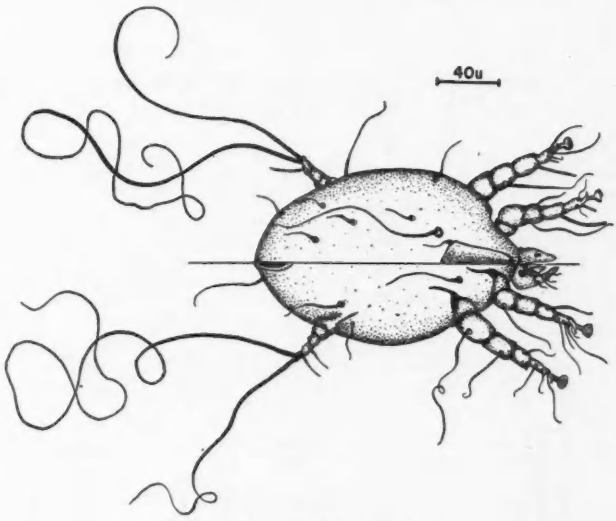


FIG. 3. Dorsoventral view of larva.

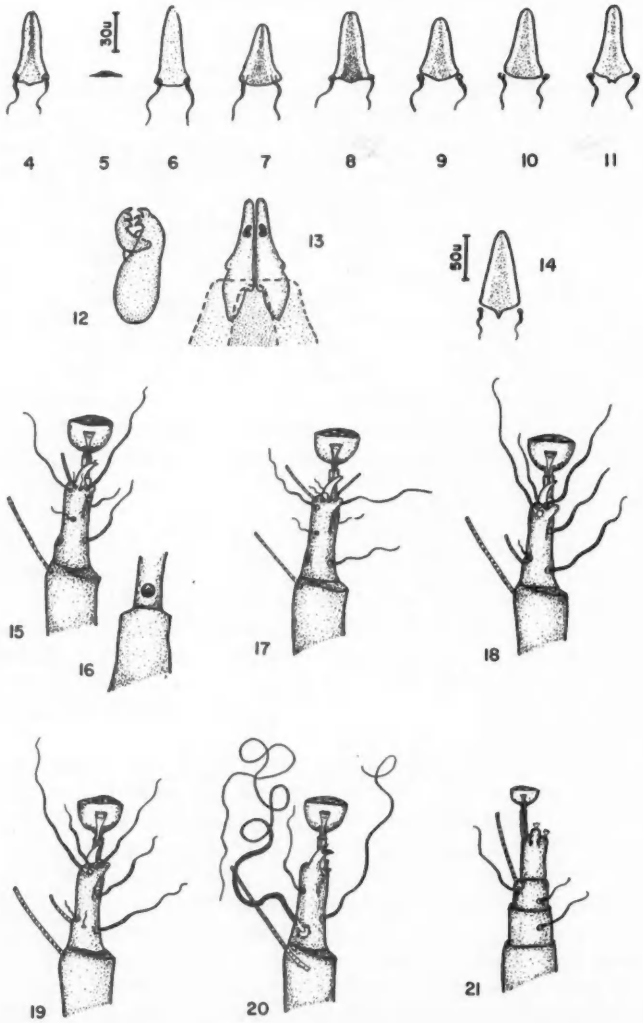
Venter

Integument finely striated except for a clear, non-sclerotized area around the posterior anus. Only four pairs of setae present—one pair of propodosomal 34–60 (42) (8), two pairs of metapodosomal, and one long pair, 71–118 (101) (13), arising terminally from the opisthosoma.

Legs

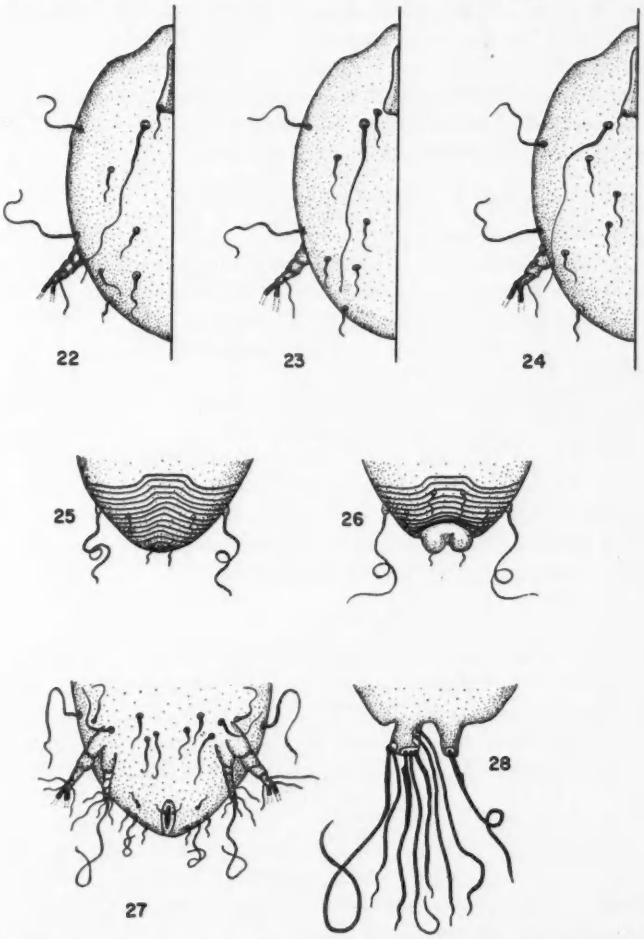
Legs I and II with six articles each, ambulatory, and approximately the same length, being 74–101 (88) (10) and 77–101 (87) (10) respectively.* Leg III with only four small, weak articles, mainly tactile, and measuring 37–54 (45) (4).† Coxae of all legs specialized as apodemata. Setae of legs I and II both long and moderately short, and increase numerically on distal articles—absent on trochanters; femurs I and II each with a single seta; genu with two setae each; tibiae I and II with one seta and one fairly stiff rod (no setal base) each, the latter situated anterodorsally. Tarsus I (Figs. 15 and 16) with a special pit, possibly a sensory structure, in the proximodorsal position; seven setae, two of which occur anteriorly and are short and inconspicuous; one moderately long rod in the distodorsal position. This pattern persists through the male and female protonymphs. Tarsus II (Fig. 18) with five long setae and one short one, the latter occurring just anterior to a moderately short rod in the proximodorsal position. This pattern persists through all female, but none of the male, stages. Tarsi I and II with a single claw and an unjointed pedunculated caruncle. Pretarsi I and II arise as an anterior "thumb" from the tarsi (Figs. 15 and 18).

*Measured from the coxa along the anterodorsal border. Does not include caruncle.
†Measurement of only the articulating part of the leg along the outer surface.



FIGS. 4 and 6–11. Variations in shape of the propodosomal plate and positions of the propodosomal plate setae. FIG. 5. Cross section of a propodosomal plate. Fig. 12. Lateral view of chelicera. FIG. 13. Dorsal view of chelicerae. FIG. 14. Propodosomal plate of adult male or ovigerous female. FIG. 15. Lateral view of tarsus I and pretarsus I of larva, male protonymph, and female protonymph. FIG. 16. Dorsal view of proximal part of tarsus I. FIG. 17. Lateral view of tarsus I and pretarsus I of male deutonymph, pubescent female, and adult male. FIG. 18. Lateral view of tarsus II and pretarsus II of larva, female protonymph, pubescent female, and ovigerous female. FIG. 19. Lateral view of tarsus II and pretarsus II of male protonymph, male deutonymph, and adult male. FIG. 20. Lateral view of tibia III, tarsus III, and pretarsus III of adult male. FIG. 21. Terminal end of leg IV of adult male.

Leg III, the last article of which has three short setae and also two extremely long whiplike setae situated distally. Of the two, the posterior is longer, 462–654 (558) (64), than the anterior, 200–312 (261) (38).



FIGS. 22–24. Irregular positions of idiosomal setae on larvae. FIG. 25. Striated opisthosomal region of dorsum of male protonymph. FIG. 26. Unstriated opisthosomal region of dorsum of female protonymph with pair of posterior suckers. FIG. 27. Ventral hysterosomal asymmetry on a male deutonymph. FIG. 28. Malformation of opisthosomal lobes of an adult male.

Gnathosoma

Chelicerae dorsal (Fig. 13) measuring 32–40 (36) long; wide, tapering apically; chelate, powerful chewing apparatus (Fig. 12); movable digit articulates by its posterior extremity in a cleft of the fixed digit; each possesses three hooked teeth on different planes. Pedipalpi located at the lateroventral

surface of gnathosoma. Possess three strong articles which gradually shorten and taper distally. Pedipalpi curve medially giving the gnathosoma a blunt appearance. They reach to the distal margin of the genu of leg I. Palpal base without setae. First palpal article with a single seta while the middle and terminal articles have two setae each. One of these, on the anterior article, is located terminally and is short and inconspicuous. The hypostome, which forms the ventromedial wall of the gnathosoma, has one pair of moderately long, centrally located setae. The gnathosoma is essentially the same in all stages, except for an increase in relative size.

Male Protonymph (Figs. 29, 12–13, 15–16, 19, 25)

(Measurements presented as for larva.)

Idiosoma and gnathosoma essentially the same as in larva, except that the protonymph is larger and possesses the fourth pair of legs. The mite is soft-bodied, and the color persists as brownish-gray.

Dorsum

Length 188–280 (240) (29) and width 140–209 (178) (23). Additional sclerotization occurs as a platelet over trochanter I. Length of propodosomal plate 55–71 (64) (5) and the accompanying setae 24–40 (33) (5). The long dorsal setae measure 110–171 (146) (17), while the lateral propodosomal measure 36–56 (42) (6) and the lateral metapodosomal 79–132 (106) (18). Six pairs of other idiosomal setae, the additional pair beyond the five pairs on the larva being in the medioterminal position.

Venter

The four pairs of setae in the larva occur in this stage, together with an additional five pairs of short setae in the hysterosomal region. The ventral propodosomal measure 37–61 (50) (10). One of the additional pairs of setae occurs near coxa IV, one pair is anal, another adanal, and two pairs flank the terminal opisthosomal setae. This last pair measures 82–150 (119) (26).

Legs

Leg I 90–126 (107) (12) long; leg II 95–128 (110) (12) long; and leg III 44–61 (52) (5) long. Terminal setae on leg III measure 258–341 (307) (26) and 575–678 (637) (41) for the anterior and posterior respectively. These legs are structurally identical with the larval legs, with the exception of tarsus II which has an additional inconspicuous seta located proximally in the laterodorsal position (Fig. 19). This new tarsal pattern persists through all male stages. Leg IV measures 21–31 (25) (3).* Composed of four articles that decrease in size apically. The final article has two short setae and one long terminal seta that measures 180–246 (212) (25).

Gnathosoma

Essentially as in the larva, except for increase in size. Chelicerae 47–52 (50)

*Measurement of the articulating leg along the inner surface.

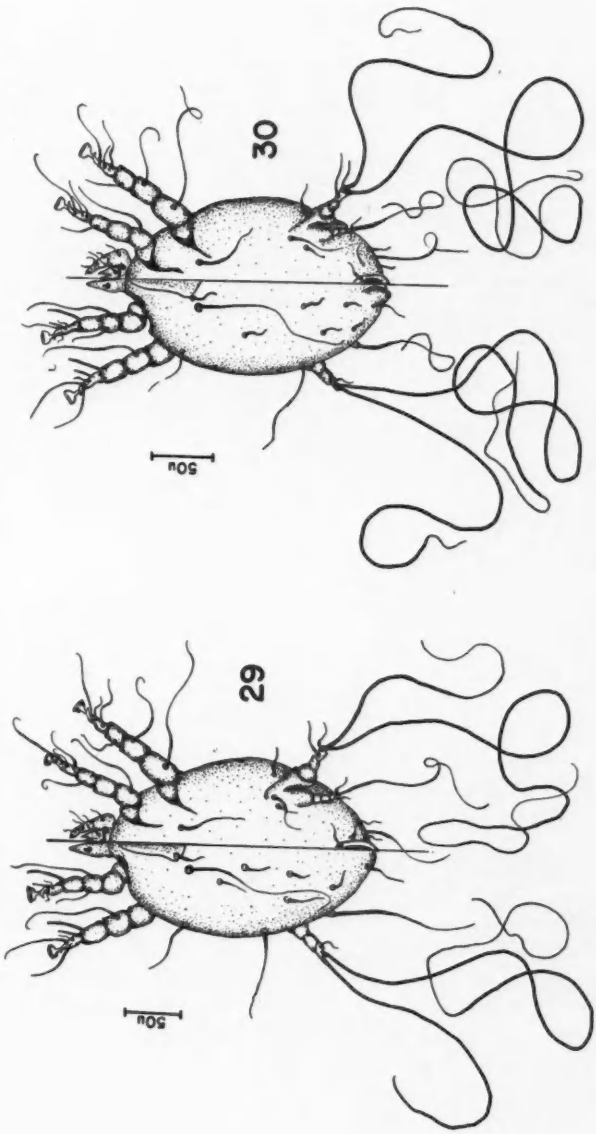
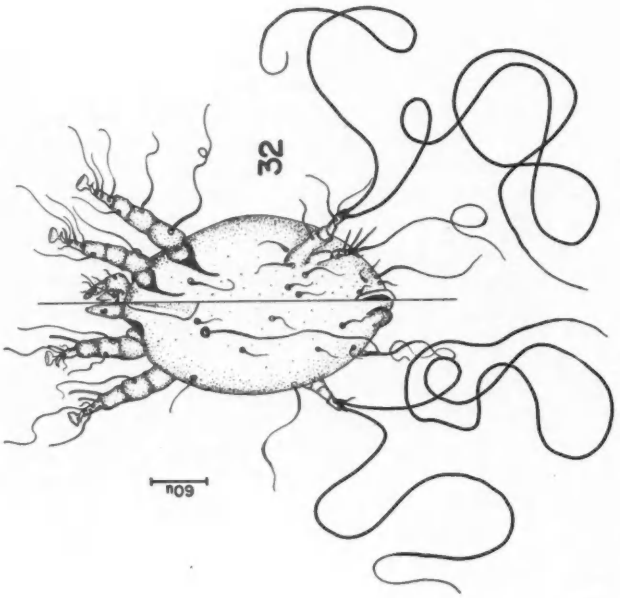


FIG. 29. Dorsoventral view of male protonymph. FIG. 30. Dorsoventral view of female protonymph.



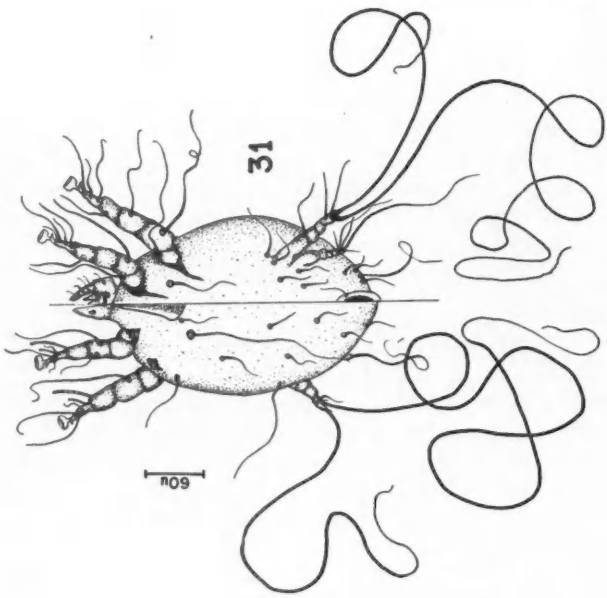


FIG. 31. Dorsoventral view of male deutonymph. FIG. 32. Dorsoventral view of pubescent female.

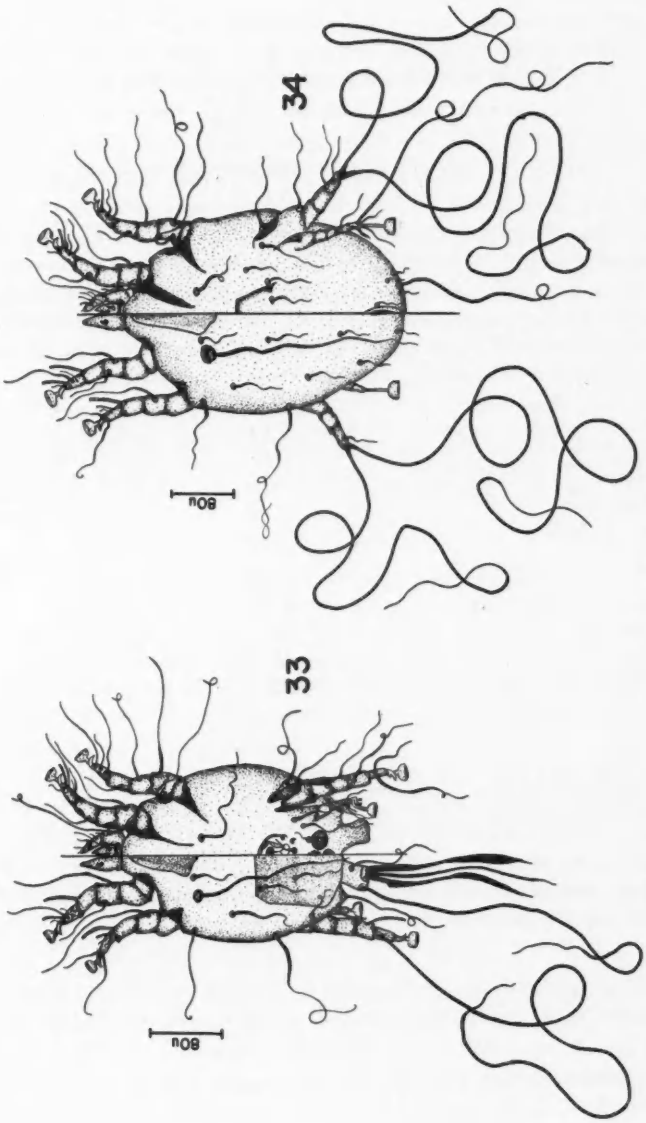


FIG. 33. Dorsoventral view of adult male. FIG. 34. Dorsoventral view of ovigerous female.

Female Protonymph (Figs. 30, 26, 12–13, 15–16, 18)

(Measurements presented as for larva.)

This stage is much the same morphologically as the male protonymph, with the additional feature of a pair of posterodorsal suckers. Tarsus II, however, unlike that of the male protonymph, remains identical with tarsus II of the larva.

Dorsum

Length 176–285 (214) (30) and width 145–238 (184) (28). Length of propodosomal plate 57–76 (63) (5) and the accompanying setae 24–35 (30) (4). The large propodosomal measure 103–142 (125) (13) while the lateral propodosomal measure 36–49 (42) (4) and the lateral metapodosomal 72–97 (81) (8). The remaining idiosomal setae are identical with those on the male protonymph. A pair of suckers occurs at the tip of the opisthosoma surrounded by a clear unstraited area (Fig. 26) that may be compared with the striated area in the same region of the male protonymph (Fig. 25).

Venter

Identical with that of the male protonymph. The ventral propodosomal setae measure 34–67 (50) (10), and the long opisthosomal setae measure 74–109 (94) (11).

Legs

Leg I 94–111 (104) (5) long; leg II 95–116 (104) (7) long; and leg III 37–55 (48) (6) long. Terminal setae on leg III measure 253–325 (285) (28) and 501–683 (611) (60) for the anterior and posterior respectively. These legs are otherwise identical with the larval legs. Leg IV measures 19–34 (25) (4) long. It is identical with leg IV of the male protonymph. The terminal setae measure 140–240 (198) (35).

Gnathosoma

As for preceding stages. Chelicerae 47–53 (49).

Male Deutonymph (Figs. 31, 12–13, 17, 19, 27)

(Measurements presented as for larva.)

This stage still displays immature characters, adding only the occasional seta and an additional article to leg IV.

Dorsum

Except for size differences and the occurrence of a second sclerotized platelet over trochanter II, the dorsum is identical with that of the male protonymph. Length 230–336 (275) (28) and width 165–250 (203) (28). Length of propodosomal plate 58–80 (74) (7) and the accompanying setae 35–48 (40) (4). These setae in this stage, the pubescent female and both adults, are never attached directly to the propodosomal plate, as is sometimes true in more immature stages. The dorsal propodosomal setae measure 153–192 (166) (12), while the lateral propodosomal measure 50–68 (57) (5) and the lateral metapodosomal 104–157 (122) (14).

Venter

Five metapodosomal setae on this mite compared with three on the protonymph readily distinguish the two stages. Other setae identical for both stages except in size. The ventral propodosomal setae measure 48–72 (62) (8), and the long opisthosomal setae measure 138–198 (164) (18).

Asymmetry is a not uncommon abnormality among invertebrates. Fig. 27 shows an example of a male deutonymph where the ventral metapodosomal setae varied in relative position on the two sides of the animal.

Legs

Legs I and II 101–134 (122) (10) and 100–136 (124) (10) long respectively. Trochanters I and II have developed one long seta each. Femur, genu, and tibia identical with preceding stages. Tarsus I has an anterior rod (Fig. 17) in addition to the structures on the protonymph. Tarsus II, however, remains unchanged. Leg III 58–77 (67) (6) long. The proximal article has acquired a seta, but the terminal article setae remain unchanged. The long terminal setae measure 304–420 (351) (36) and 690–865 (786) (57) for the anterior and posterior respectively. Leg IV measures 30–40 (36) (3). Now composed of five articles that decrease in size apically. The fourth article has one seta, and the apical article four setae, of which the terminal measures 293–364 (331) (23).

Gnathosoma

As for preceding stages. Chelicerae 52–58 (55).

Pubescent Female (Figs. 32, 12–13, 17–18, 38)

(Measurements presented as for larva.)

General appearance of the female protonymph dorsally and the male deutonymph ventrally.

Dorsum

Identical with that of the female protonymph. Like this, but unlike the male deutonymph, there is no platelet over trochanter II. Length of dorsum 258–361 (288) (30) and width 180–246 (222) (31). Length of propodosomal plate 61–84 (73) (7) and the accompanying setae 27–34 (31) (3). The dorsal propodosomal setae measure 130–314 (220) (45), while the lateral propodosomal measure 30–45 (38) (5) and the lateral metapodosomal 66–81 (75) (5).

Venter

Identical with that of the male deutonymph. The ventral propodosomal setae measure 32–61 (46) (10), and the long opisthosomal 106–248 (178) (43).

Legs

Four legs much like those of male deutonymph; only exception is tarsus II which is identical with that of the female protonymph. Leg I measures 97–119 (103) (10); leg II 95–115 (101) (6); leg III 36–53 (42) (5); and leg IV 27–32 (30) (2). The whiplike setae of leg III measure 233–327 (293) (27) and 534–669 (593) (53) for the anterior and posterior respectively. The terminal seta of leg IV measures 151–195 (175) (15).

Gnathosoma

As for other stages. Chelicerae 47–56 (53).

Adult Male (Figs. 33, 12–14, 17, 19–21, 28, 38)

(Measurements presented as for larva.)

Marked morphological changes exist between the male deutonymph and adult. Additional sclerotization occurs on the dorsum, venter, and legs. All legs have caruncles.

Dorsum

Length 272–325 (294) (16) including opisthosomal lobes. Width 208–238 (228) (9). The propodosomal plate is often pointed posteriorly (Fig. 14) and measures 72–85 (79) (4). Many of the setae that occur on the immature stages are present. The two most posterior pair of setae on the deutonymph, however, are replaced in the adult by a moderately long pair of opisthosomal setae and two opisthosomal lobes which also support setae. The pair of setae near the propodosomal plate measures 43–53 (50) (3), and the dorsal pair of propodosomal measures 172–217 (194) (16). The lateral propodosomal measures 71–87 (80) (5) and the lateral metapodosomal 198–253 (229) (20). Additional sclerotization is present in the form of a large septagonal plate over much of the hysterosoma, while the platelets over trochanters I and II persist as on the deutonymph. A pair of large opisthosomal lobes occurs which supports five pairs of setae. Three of these arise from the posteromedial corner of the lobe from a common setal base. The middle seta of the three is thick basally but gradually tapers distally; the other two are also thick basally, and towards the distal end change to a membranous ensiform or lanceolate shape. The outer of the three also has a conspicuous hook towards the proximal end. Another thick seta arises from a large setal base at the outer corner of the lobe. A more typical seta also arises from an anteromedial position of the lobe.

Malformations, like the asymmetry noted previously, sometimes occur among lower animals. Fig. 28 shows an anomaly in the opisthosomal lobes of an adult male that was apparently in normal attachment with a female. The male appeared otherwise normal. One opisthosomal lobe supports only a single seta, while the other supports the usual complement of five with the addition of two others, each of which arises from a setal base supporting a normal seta. All the setae, unlike those on normal mites, taper gradually to a point.

Venter

Like the dorsum, the hysterosomal region of normal adult males has changed markedly between the deutonymph and adult. Just anterior and lateral to the anus, a pair of adanal copulatory suckers occurs with an associated pair of short setae. In the center of the metapodosomal region the reproductive apparatus is now present together with a pair of setae and two pairs of hooks. The deutonymphal pair of anal and adanal setae, as well as one pair of metapodosomal setae, do not occur on the adult. The ventral propodosomal setae measure 81–103 (88) (7).

Legs

Apodemata heavier and more extensive than on immature stages and coxae II and III with some additional sclerotization. Legs I and II 143–162 (157) (8) and 145–177 (160) (9) long respectively. Identical with those of the deutonymph with the addition of some sclerotization on genu I and tibia I. Leg III 150–166 (160) (5) long and now has five articles. The long whiplike setae of the deutonymph do not occur, but instead there is a tarsus with three setae, a caruncle, a hook with two teeth, and a third ventral hook in the same relative position as pretarsi I and II. The tibia supports a rod and seta (Fig. 20). Leg IV 61–74 (69) (4) long and now with five articles. The long terminal deutonymphal seta is absent, but there is now a tarsus with three small distal lobes (Fig. 21), two of which support a minute caruncle each, while the third gives rise to a larger caruncle. The latter, however, is only about half the size of those on tarsi I, II, and III. The tibia supports a rod and two setae.

Gnathosoma

As for other stages. Chelicerae 40–58 (53).

Ovigerous Female (Figs. 34, 12–14, 17–18, 39)

(Measurements presented as for larva.)

Morphologically, the ovigerous female does not change so drastically from the immature stages as the adult male. The genital suckers do not occur dorsally, but the vulva is present ventrally. Leg III, unlike the adult male, retains the terminal whiplike setae that occur on immature stages.

Dorsum

Much like that of the pubescent female—same setae, but without the posterior genital suckers. With sclerotized platelets over trochanter II and around the setal base of the dorsal pair of propodosomal setae. Dorsum 306–415 (358) (30) long and 229–287 (248) (18) wide. Length of the propodosomal plate (many are pointed posteriorly as in adult male) 92–107 (98) (5) and the accompanying setae 37–47 (42) (3). The dorsal propodosomal setae measure 185–229 (203) (15), while the lateral propodosomal measure 43–64 (56) (6) and the lateral metapodosomal 129–158 (140) (8).

Venter

Vulva present in central podosomal region; consists of transverse and diagonally posterior slits, and hinges posteriorly. Venter with one less pair of metapodosomal setae than pubescent female. The ventral propodosomal setae measure 56–71 (65) (5), and the long opisthosomal 194–257 (223) (22).

Legs

Apodemata heavy and extensive and with some associated sclerotization on all legs. Darker color than those of pubescent female. Legs I and II 138–164 (154) (9) and 125–167 (155) (12) long respectively. Identical with those of pubescent female. Leg III 87–106 (100) (6) long and of four articles

like the pubescent female, but unlike the adult male. The whiplike setae measure 377–542 (449) (45) and 744–886 (835) (37) for the anterior and posterior respectively. Leg IV 100–129 (118) (11) long; terminal article with a caruncle and also one quite long and three moderately short setae.

Gnathosoma

As for other stages. Chelicerae 58–72 (65).

Egg (Fig. 44)

(Measurements presented as for larva.)

The eggs are elliptical and have white, shiny surfaces. Length 139–181 (164). Shell has two bosses (Fig. 44) on the same side and towards one end of the egg which, during embryonation, is the capital end of the egg.

B. Life History

Since chorioptic mites live on the skin, it would be desirable to learn the mode and kind of their general nutrition. Fig. 12 shows that these mites have chewing chelicerae. Material that they eat is excreted in the form of pellets, some of which accumulate about the anus. Food in the digestive tract is passed from one active stage to the next via the intermediary quiescent stage; and the new stage, when starved, will excrete pellets from the former stage. In culture, mites were maintained as successfully on epidermal debris and hair kept 4 months before use, and on material sterilized by heat, as on freshly obtained material. Therefore, the mites can, and on the host probably do, feed on dead tissue. Washed hair from cattle was unsuitable, whereas epidermal material and hair obtained from an Indian water buffalo, which had hair, resembling wire, that was presumably unpalatable, was highly satisfactory. These results showed that it was the epidermic debris, and not the hair, on which the mites fed. This verified Fürstenberg's belief of 1861 that the mites lived on sloughed epidermis, a suggestion found unacceptable in 1872 by Mégnin (15), who was responsible for influencing some later observers.

In the life cycle, it is the larval stage that follows the egg, but it will be understood that the larva is actually formed before the egg hatches. Larva is defined here as that period from the hatching of the egg up to its ecdysis into the protonymph. It includes, therefore, the quiescent period immediately preceding ecdysis during which internal transformation takes place from the larval state *per se* to the protonymphal state. Terms for the subsequent stages will be used for the period from one ecydsis to the next.

To observe a stage in the life cycle, eggs or quiescent mites were transferred directly from the host or from a culture vial to an unused vial. Only one or a few eggs or quiescent stages were used at a time. Epidermic debris and hair from a positive area on the hind legs of a grade Holstein–Friesian cow were then added to the vial, but only after the material had been heated to 400° F. This killed the mites that were collected with the epidermal material. Observations were made on the biology and longevity of the stages that emerged.

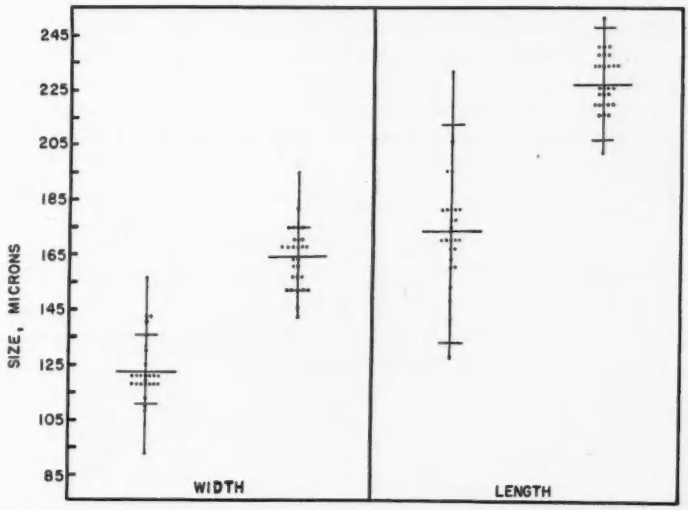


FIG. 35. Width and length of larvae at the time of hatching and at quiescence. Each point is an observation, and the vertical line marks the range. The long horizontal bar represents the mean and the short horizontal bar represents twice the standard deviation of individuals.

The larva is an active feeding stage. If starved, it will not live more than a day. A feeding larva gradually increases in size from the time it hatches until it enters quiescence. The actual amount of increase was determined by comparing the length and width of recently hatched and unfed larvae with the same measurements for quiescent larvae. Both length and width increased by approximately a quarter (Fig. 35). Under the *in vitro* conditions of 35° C. and 80% R.H., the larva generally remained motile for 3 to 5 days, but sometimes for as long as 12 days (Table I) after hatching from the egg. Since the eggs took 4 days to incubate, this was 7 to 16 days following oviposition. The mite then enters quiescence. Some larvae lived longer than 12 days, but these failed to become quiescent and died as larvae. Healthy larvae spent 22 to 30 hours in the quiescent state. This wide variation under such constant conditions may be related to the time required by the protonymph to split the larval integument, rather than on the period required for the complete transformation process. Only the occasional quiescent mite failed to molt. The new stage is formed during quiescence when histolysis of the internal tissues occurs. The tissue within the legs is resorbed into the body of the larva, leaving the legs of the latter as hollow exoskeletons. The mite, therefore, loses its motility completely. It maintains its position on the host or on the glass of the vial, however, by the suction of the caruncles and to a lesser extent by the claws on the tarsi which remain with the old exuvium. Nevertheless this attachment is not firm. Oftentimes the quiescent mite was observed on its back or in other unoriented positions, but this did not seem to interfere with the transformation process. Quiescent mites of

various stages were frequently clustered together on the underside of a piece of scab, or with a group of eggs, or elsewhere. This suggests that some mites secure a common, and sometimes protected, location just prior to the transformation period. It seems likely that at least some immotile mites on cows, particularly those without scabby lesions, must be brushed off the host during quiescence.

TABLE I

LONGEVITY OF IMMATURE STAGES

Period, days	Motile period				Period of attachment pair
	No. of larvae	No. of male protonymphs	No. of male deutonymphs	No. of female protonymphs	
2	–	–	–	–	2
3	22	19	24	14	6
4	32	21	16	11	12
5	12	8	5	2	10
6	2	3	2	1	15
7	1	1	1	–	14
8	1	–	–	–	9
9	2	1	–	–	–
10	4	–	–	–	–
11	1	–	–	–	–
12	1	–	–	–	–

Immediately preceding ecdysis, it is possible to discern the protonymphal characters. Fig. 36 demonstrates this. A space is apparent between the larval integument and that of the protonymph. The protonymphal gnathosoma and pedipalpi develop immediately behind the larval gnathosoma, and like the legs, within the exoskeleton of the larval body. Legs I and II are folded posteriorly over the venter of the protonymph, while legs III and IV with their long setae project over the venter anteriorly. Molting is a relatively simple process. The new stage ruptures the larval integument transversely across the mid-dorsal hysterosoma. This is followed by a mediodorsal longitudinal rupture extending from the transverse slit forward to the propodosomal plate. The integument then rolls laterally in both directions along the posterior edge of the propodosomal plate. The new stage emerges by backing out of the exuvium between the slits. The complete exoskeleton with the setae and propodosomal plate is discarded. The pattern is the same for all stages, and the positions of the slits on a more advanced stage are shown in Fig. 37.

Two morphologically distinct protonymphs representing each sex (Figs. 29 and 30) emerge from the larval stage. Both are active feeding stages which gradually increased to a maximum size, and most entered quiescence after 3 to 5 days. Many protonymphs that lived longer than that period died as protonymphs, although a few did enter quiescence and did eventually molt (Table I). The protonymphal quiescent period, like that of the larva, lasted 22 to 30 hours. The method of emergence of the male deutonymph or

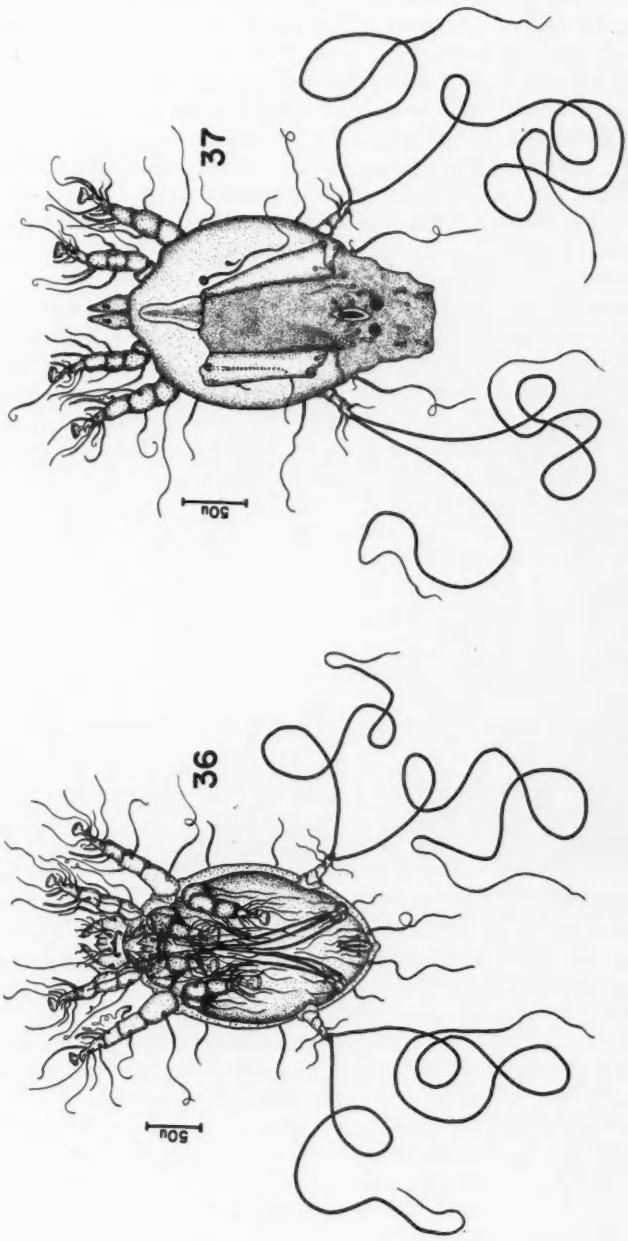
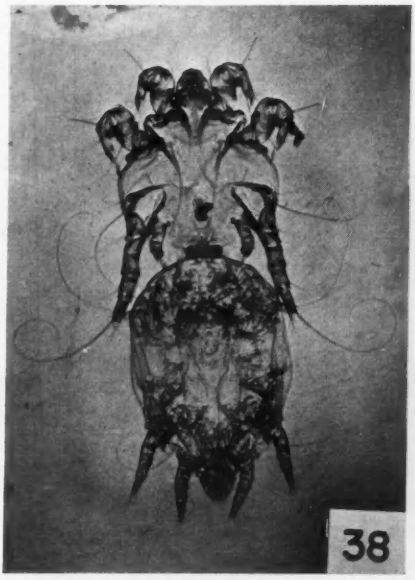


FIG. 36. Quiescent larva containing a developed male protonymph. FIG. 37 Molt skin of a female protonymph.

the pubescent female was also identical with that of the previous stage. Like both preceding stages, the male deutonymph was an active creature and generally remained motile for 3 to 5 days, then entered quiescence and molted into the adult male in the same manner as the other stages. Upon emergence, the adult male moved rapidly through the hair and epidermic debris until it found a pubescent female with which it became immediately attached, primarily by means of its copulatory anal suckers (Fig. 38). This is the attachment pair, or what previous authors referred to as the copulation pair. Only a few adult males failed to become attached to pubescent females when the latter were available. The attachment period generally persists through both the motile and quiescent periods of the pubescent female. Table I shows that this might be 2 days, but is generally at least 4 days and frequently 7 or 8 days. The pubescent female, therefore, generally lives twice as long as the other immature stages. During the attachment period, the male drags the female wherever it goes. It is rare for the mites to terminate their attachment spontaneously either permanently or temporarily. Pairs separated mechanically seldom reunited. Normal attachment is so firm that it is often continues after one member of the union has died; and the male is frequently left, following the female's molt, pulling an empty exuvium. The adult male, which is blind, sometimes attaches itself to various stages including the female protonymph (which has posterior attachment suckers), the ovigerous female, or even another adult male. Unattached pubescent females that either were in the presence of single adult males or were reared separately from protonymphs never entered quiescence or made the final molt, but persisted in the motile condition frequently for 2 to 3 weeks and on two occasions for 30 days. A continuance in the motile state would assure a greater chance of attachment. Once in attachment with males *and* once the pubescent females have undergone quiescence, it is then possible to pinch off the males without interfering with the final molt of the females. These females, however, do not lay eggs. Nor will these females begin laying after being exposed in their final stage to adult males. These observations demonstrate that the act of copulation, however accomplished, takes place with the ovigerous female at a precise moment during the final molt of the female, and that the long period of attachment simply makes certain that the male is available at the appropriate moment for copulation. It demonstrates also that eggs are not laid parthenogenetically.

Unlike the preceding stages for which the longevity was determined by starting with either eggs (for the larva) or quiescent stages (for the nymphs), the total period required for the development of adult males, ovigerous females, and the laying of the first egg were determined (frequently independently) by starting with large numbers (30 to 200) of eggs 0 to 24 hours old. Ovigerous females were first seen between the wide limits of 16 to 26 days following oviposition, but most occurred after 19 to 23 days (Table II). The females increased in size to a maximum during a preoviposition period of 2, but sometimes 3 or 4 days. They then began to lay a series of eggs without additional intervention by a male. The first egg to be laid,



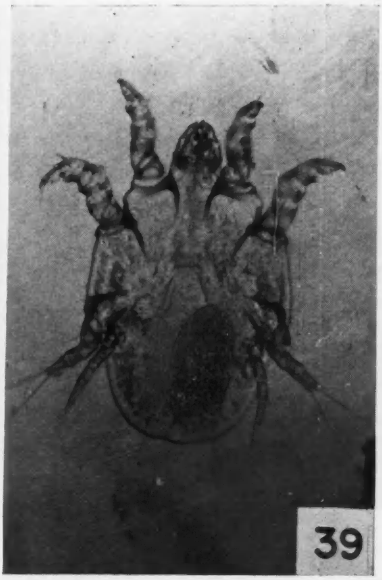


FIG. 38. Photomicrograph of an attachment pair. Adult male above and pubescent female below. FIG. 39. Photomicrograph of an ovigerous female containing an egg.

concomitant with the first appearance of the ovigerous females, varied widely between 18 and 28 days after the original oviposition. Table II shows that many took 21 to 26 days. This 3-week period was, therefore, that usually required for a complete life cycle under the *in vitro* conditions.

It will have been noted from Table I that a summation of the minimum periods of survival for the larva and two male nymphs plus the incubation time of the egg (4 days, but sometimes 3) and three quiescent phases (1 day each) comes to 15 to 16 days. This was observed to be true in vials started with numerous eggs, although most adult males emerged from 17 to 20 days after oviposition (Table II). Many pubescent females took 13 to 14 days to appear. Since this is at least 1 and generally 2, 3, or more days before adult males occurred, and since the latter generally became attached to pubescent females immediately after emergence from their previous stage, it is most probable that on the host, members composing an attachment pair are from eggs laid at different times.

TABLE II

PERIOD FOR ADULT MITES AND NEXT GENERATION

From oviposition, days	No. of adult males	No. of ovigerous* females	First egg
15	4	–	–
16	7	4	–
17	20	6	–
18	19	3	2
19	13	14	4
20	16	8	6
21	9	19	8
22	6	10	6
23	2	11	10
24	–	4	10
25	–	2	7
26	–	3	15
27	–	–	6
28	–	–	2

* The ovigerous females are not necessarily the same individuals that produced the eggs listed in the final column.

Ovigerous females demonstrated oviposition periods of 3 to 16 days (Table III). Each female laid eggs at the rate of about one a day. The occasional female laid two eggs, and an odd female three eggs, in a 24-hour period. This rapid reproduction, when it occurred at all, was invariably at the beginning of the oviposition period. Following oviposition, there was a period of senescence that generally lasted 3 days, but ranged from 1 to 5 days (Table III). Non-productive adult females outlived their egg-laying counterparts. While the latter lived up to 3 weeks, most of the former lived up to a month and few as long as 49 and 55 days. The longevity of adult males, whether they had been in attachment with females or not, corresponded to that of the non-laying females by living up to 49 days.

TABLE III

EGG PRODUCTION OF OVIGEROUS FEMALES RAISED INDIVIDUALLY

Preoviposition period, days	No. of females	Oviposition period, days	No. of females	Total eggs laid by a single female	No. of females	No. of egg groups*	No. of females	Senescence period, days	No. of females
2	33	3	3	3	3	3	6	1	5
3	18	4	4	4	3	4	7	2	12
4	15	5	4	5	5	5	17	3	38
		6	3	6	5	6	10	4	8
		7	6	7	6	7	6	5	3
		8	8			8	3		
		9	7	9	8	All laid singly	17		
		10	6	10	9				
		11	6	11	10				
		12	4	12	6				
		13	3	13	4				
		14	8						
		15	2	15	2				
		16	2	16	3				
				17	2				

* Number of eggs in each group ranged from one to five.

Females lay their eggs singly. The act of laying takes but a minute or two. With the egg's expulsion, there is a simultaneous secretion of a fluid that lubricates the egg in its passage, and which also, upon solidification, firmly attaches the egg to the substrate by its end or along one edge. On the host, the substrate is a flake of skin scurf contiguous with the animal's body, or the outer layer of skin, and in the culture vials it is the surface of the glass. One female, though blind, tends to return to a few places to oviposit, although no female ever lays all its eggs in only one or even two groups. Table III shows that some females, raised individually, used from three to eight sites to deposit 3 to 17 eggs with up to five eggs in a group, but many, under these isolated conditions, chose a different location for each egg. A few females deposit some of their eggs in an apparently haphazard fashion amongst the hair and debris in the culture vials. The exact number was determined by subtracting the number of eggs attached to the substrate from the total number of larvae recovered following incubation. The difference indicated the number of eggs laid in the hair, which for each of 10 culture vials, ranged between 22% and 36% of the 9 to 11 eggs laid.

In vials with numerous mites, the presence of eggs from one female attracted other females to lay their eggs at the same site, resulting in communal groups. Groups of eggs often began at the peripheral margin of the bottom of the vials, and spread gradually inwards towards the center, or up the sides of the vial. Less frequently, groups of eggs were produced in more central positions of the bottom of the vial. When numerous females were present, the number of eggs in a group usually ranged from three to four to a few hundred, but one group contained 2961 eggs which spread over an area of 3.7 sq. cm. All eggs were spread out singly in close proximity over the surface of the substrate and were only rarely laid *in cumulo*. Because of this, each egg was exposed to virtually the same microclimate. Hence, following incubation, eggs hatched in the same sequence as they had been laid.

Some observations were possible on the development of the egg during both pre- and post-oviposition. Preovipositional development consisted of the formation of the yolk mass in the hysterosomal region of the ovigerous female. A small mass in irregular outline was first apparent on either the left or right side of the mite. This enlarged irregularly (Fig. 40), but eventually acquired a regular oval outline (Fig. 41). It may be enclosed by a membrane. With further enlargement, the yolk mass assumes an elliptical shape, typical of the egg, and is enveloped by a membrane. The shell, however, has not yet formed (Fig. 42). When this stage is reached in some, but not all, mites, a second yolk mass becomes apparent on the opposite side of the female. The latter increases into an irregular yolk mass at about the same time that the original egg acquires its shell (Fig. 42). The egg then moves medially to a position posterior to the vulva (Figs. 39, 43). From these observations, it seems likely that eggs develop in an alternating bilateral fashion. This would partly explain why some mites could lay two or even three eggs in a day while most laid only a single egg in that period.

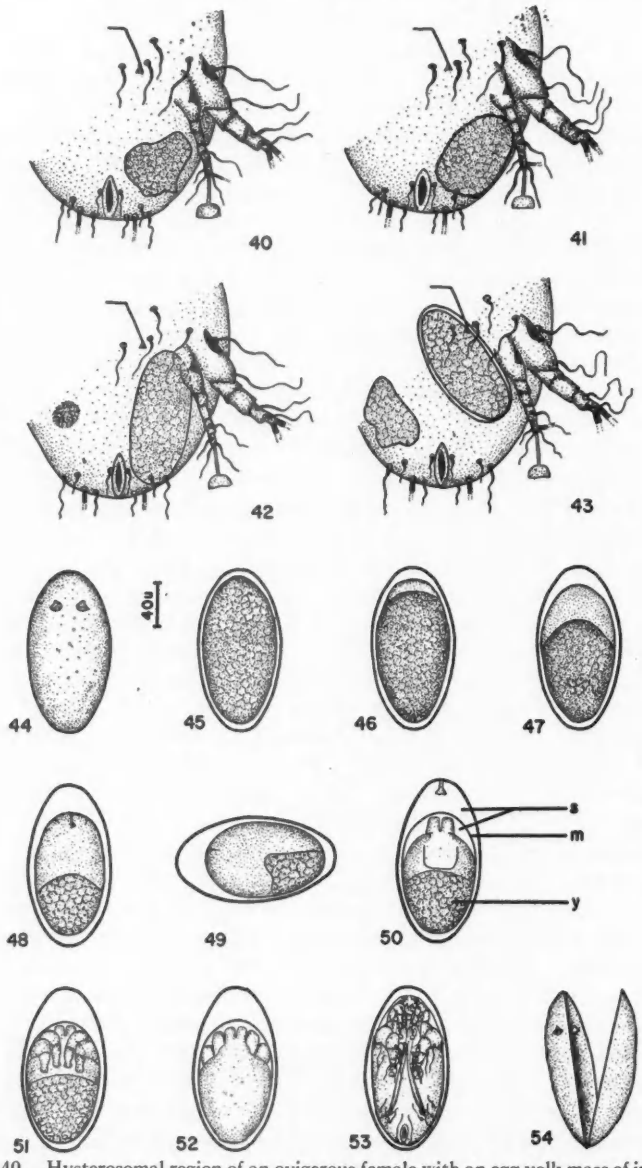


FIG. 40. Hysterosomal region of an ovigerous female with an egg yolk mass of irregular outline. FIG. 41. The same region with an egg yolk mass of an oval shape. FIG. 42. The same region with an egg yolk mass of an elliptical shape. Another small egg yolk mass appears on the opposite side of the female. FIG. 43. The same region with a shelled egg just posterior to the vulva, together with another enlarged egg yolk mass. FIG. 44. Egg shell with two bosses. FIG. 45. State of egg at time of laying. FIGS. 46–48, 50–51. Degree of embryonation after about 2, 5, 10, 16, and 48 hours respectively. *s* = space; *m* = membrane; *y* = yolk mass. FIG. 49. Lateral view of Fig. 48. FIG. 52. Dorsal view of Fig. 51. FIG. 53. Complete development of larva within egg. FIG. 54 Discarded egg shell.

Many eggs, like the one behind the vulva in Fig. 43, had been laid by some females (Fig. 45). Postovipositional development was observed in eggs collected within 2 to 3 hours following oviposition. The eggs were partially cleared and examined in glycerine in a depression slide. Within the first 2 hours, the yolk mass had partly receded from the capital pole, displaying a translucent egg white area (Fig. 46). After about 5 hours, the latter had extended to about a quarter of the length of the egg (Fig. 47). The yolk mass occupied less than half the posteroventral part of the egg about 10 hours after oviposition when there also appeared three indentations in the egg white at the capital end (Figs. 48 and 49). The primordial mouth parts are first to appear in the form of a pair of anterior projections, still enveloped within a membrane (Fig. 50). This occurs after approximately 16 hours. After about 48 hours, legs I and II appear as partially differentiated projections (Fig. 51) arising from distinct shoulders (Fig. 52), and are directed posteriorly over the venter. (Leg III may be present and concealed by the yolk mass.) Near the end of the incubation period, all the yolk mass is apparently absorbed, and complete differentiation of the larva has occurred within the egg. A space occurs between the larval integument and the egg-shell. The larval gnathosoma and pedipalpi are pointed anteroventrally, and the legs, like those within quiescent mites, lie over the venter of the larva with the anterior legs directed posteriorly and the hind legs are directed anteriorly with their setae extending to the tip of the gnathosoma. Because of their length, the setae must then turn posteriorly, but this could not be traced (Fig. 53). Each larva emerged through a cleavage of the shell that encircled the egg along its longitudinal axis. Both bosses on the egg shell were on the same side of the cleavage (Fig. 54). Sometimes the two halves of the egg remained unseparated at the posterior end or along the edge attached to the substrate, but this did not interfere with the emergence of the larva.

Under the *in vitro* conditions, the postoviposition period required before the eggs hatched varied from 72 to 107 hours, but most eggs took 90 to 95 hours (4 days) to hatch (Table IV). Larvae emerged anterior end first. Only three eggs among thousands failed to hatch. Two of these did not differentiate, while the third contained a complete larva that failed to split the shell. Why some eggs took longer to hatch than most may not be related to the degree of embryonation but to the time required to split the shell. This, in turn, would probably depend in part on the degree of attachment of the egg

TABLE IV

HATCHING TIME OF EGGS AFTER OVIPOSITION

Time, hours	No. of eggs	Time, hours	No. of eggs
72–77	6	90–95	102
78–83	8	96–101	46
84–89	10	102–107	4

with the substrate. At the other extreme, the fact that some eggs took only 3 days to hatch might be explained by previous prolonged retainment within the female. A few of the eggs examined in glycerine immediately after they had been laid had developed to the extent that some of the yolk mass had receded posteriorly. An abnormal situation occurred where a freshly killed ovigerous female contained an egg that had fully embryonated. If this egg had been laid, it would have been only a matter of a few hours before it would have hatched. (The end of this egg that pointed towards the vulva was the posterior end of the mite, but it is unknown whether all eggs are laid in that direction.) Much of the variation in the postovipositional time of hatching, therefore, may be attributable to preovipositional retainment. This would also explain some of the variation in the 2 to 4 days required for preovipositional periods (Table III).

Leg structure appears to be closely related to the reproductive activities of the mite. The ratio of the length of the mite to its width varies from 1.16 for the female protonymph to 1.44 for the ovigerous female. The value for the male deutonymph is 1.36 and for the four remaining stages remains a constant 1.30. Because of this fairly consistent relationship between length and width, one of these—length—was used as the abscissa in Fig. 55, and against it were plotted the length of the various legs in each stage. For the male stages, leg I shows an expected increase in length with an increase in

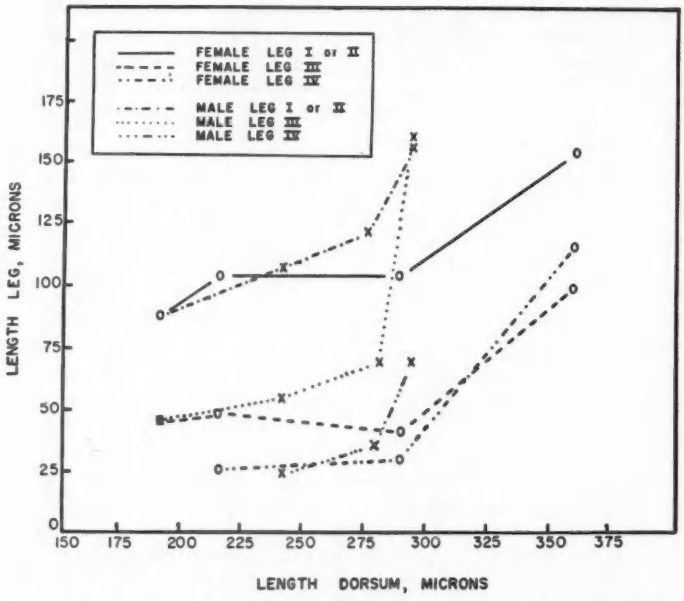


FIG. 55. Comparative increase in leg length from one stage to the next. From left to right, the first series of vertical points represent the larva; the second the female protonymph; the third the male protonymph; the fourth the male deutonymph; the fifth the pubescent female; the sixth the adult male; and the seventh the ovigerous female.

body size. The length of leg I of the female stages, however, increases from the larva to the female protonymph, but maintains the same size in the pubescent female stage, even though the body has increased in size by a little less than a third. This loss is regained by the ovigerous female where leg I is of a comparatively "normal" length. Results for leg II are virtually the same as for leg I. Leg III of the female stages shows the same graphic pattern as legs I and II. It seems possible that the comparative decrease in the length of the three anterior legs in the pubescent female reflects their limited usefulness, since most pubescent females spend this entire phase being dragged around by the adult males. Leg III of the adult male also shows a relation to what has happened in the pubescent female stage. It has an additional article and obviously greater strength than that of the preceding male stages, and has replaced the two whiplike terminal setae with a caruncle and hooks (Fig. 20). In general, the leg has transformed from one that is essentially sensory to one that is mainly ambulatory. It is the third leg that would support much of the weight of the attached female. Concomitant changes take place in leg IV. On all four of the first and second male and female nymphs, this leg is short, weak, and perhaps a little more sensory than ambulatory. Leg IV of the adult male increases considerably in length beyond that on immature stages, and is equipped with three caruncles. It seems, therefore, to be suited mainly for traction. The importance of this in support of, and making proper attachment with, the pubescent female is obvious. In the ovigerous female (where leg III persists essentially as a sensory structure) leg IV appears to support most of the weight. Such would be most important to a female in the process of egg-laying.

The foregoing observations on the life cycle have demonstrated that the structure and activities of each of the seven active stages are similar to one another, and that the wide variations in their longevity suggest that a constant temperature and humidity, as influential as they probably are, may be less significant than some unknown factors. Feeding may have an appreciable effect on the survival of each stage. This will become apparent in the following sections of this communication.

The physical environment on the hide of a cow or other ungulate may vary at different sites and between wide limits. Such would influence the period necessary for completion of a mite's life cycle. Direct measurements were made of one physical factor—temperature. By means of a calibrated contact pyrometer,* skin temperatures were taken on members of a Holstein–Friesian herd in winter when the animals were maintained in a warm, well-ventilated barn. The temperature of most body areas, including the feet where most mites generally occur, varied irregularly between 31° and 35° C. The temperature of the side of the udder, where there is considerable blood supply, was somewhat higher (36° to 37° C.), and the crotch between the udder and thigh of a high production animal was generally 37° to 38° C. The pasterns of a Jersey cow in another barn, however, had a temperature of only 30° C.

*Manufactured by Light Laboratories, Brighton, Sussex, England. Ser. No. 163.

This probably reflected the colder floor temperature and smaller leg and foot size of this breed. Hosts of other species of *Chorioptes* were examined. The body temperature of goats, the host of *C. caprae*, varied between 33° and 38° C., while the feet—where most mites were found—of members of the herd in question were so cold that no temperature would record on the pyrometer, which registers a minimum temperature of 25° C. Two Belgian horses, naturally infested with *C. equi*, had pastern temperatures of 35° to 36° C. The environment of a chorioptic mite varies, therefore, with the environment of the barn or stable, the locus of the mite on the host, and, when infesting the leg of the host, on the mass and probable blood supply of that extremity. Additionally, eggs laid *in vitro* within a period of 24 hours were placed in an isolation unit attached to the back (33° to 34° C.) of a grade Holstein cow. The eggs were separated by bolting silk from the cow's hide, but were exposed to virtually the same physical environment as eggs in a normal habitat. These took 5 to 6 days to hatch, whereas eggs under *in vitro* conditions generally took 4 days. It seems likely from the above observations that the period required for a life cycle on the host varies greatly with the conditions, and that it is generally somewhat longer than that observed under the *in vitro* conditions.

II. Non-Specificity and Revision of the Genus *Chorioptes*

During the past 100 years, eight species or subspecies of *Chorioptes* have been described from almost as many hosts. Most were on farm stock, but some were from animals in zoological gardens. In the present study, a wide variety of mammalian species and breeds were examined for chorioptic mites. Five host species were found. Studies followed on the *in vitro* culture of the mites from the different hosts, a morphological comparison of the mites, and on the validity of the species in the genus *Chorioptes*. Before describing the experimental results, it is imperative to clarify exactly what has been written by others on natural infestations on all hosts, and the criteria used by them for the creation of the different species in the genus.

Natural Infestations on All Hosts

Natural infestations on all hosts that have been noted throughout the world are summarized, irrespective of the specific names applied by the author, together with those infestations observed in the present study to ascertain any detectable difference in mite behavior on different hosts, as well as comparative data on the incidence of infestation. The literature survey showed that published reports for the most part referred to mites only because the observer was confronted with a dermatitis that required an etiological explanation. Observations on mites in the absence of a pathological condition have been scant indeed.

(*a*) *Domestic Cattle and Horses*

A review of the papers in various agricultural and veterinary bulletins suggests that chorioptic mites have a cosmopolitan distribution on cattle and horses. It is recognized generally that the dermatitis sometimes

associated with the mites usually remains localized and appears for the most part in winter. Rose (25), however, stated that the condition was more apparent on horses in summer in the warm, moist atmosphere of New South Wales. Horse breeds of purebred stock found naturally infested in the present study were the Clydesdale, Belgian, and Percheron, with some members of the first two breeds displaying typical lesions on the posterior pasterns, sometimes referred to colloquially as "Clyde itch" or "foot mange". For cattle, observations made monthly for a year on a herd of one breed (Holstein–Friesian) were noted previously (26). Various other positive cattle breeds (Ayrshire, Shorthorn, Dual-purpose Shorthorn, Hereford, Aberdeen Angus, Scotch Highland) were examined in which few of the infested individuals displayed mild skin eruptions.

(*b*) *Domestic Sheep and Goats*

Reports of infested sheep and goats have appeared less frequently than those of cattle and horses. In Germany in the last century, Zürn (28) observed a Negretti ram with chorioptic mange on the legs and scrotum. More recently, McKenna and Pulsford (13) listed only two reports of chorioptic mites on sheep in Australia, one of which included lesions on the scrota of Corriedale rams. Foot and scrotal mange associated with chorioptic mites on Corriedale and other sheep breeds may be more prevalent in New Zealand than in Australia (8, 17). Interestingly, both breeds mentioned above (Negretti, Corriedale) were bred from a merino foundation. In 1909, Cave (4) summarized four reports of infested flocks in England, one associated with foot-rot, and another in Kent (Romney Marsh) lambs with lameness. In the present study, 8 of 38 Suffolk sheep in two flocks were found with mites, but not mange, in Quebec: and chorioptic mange occurred on the muzzle and lower legs of some members of one flock of grade sheep in Nova Scotia.* Reports of chorioptic mites on goats are about as sparse as those on sheep. In 1854, Delafond and Bourguignon (5) found chorioptic mange on a number of Angora goats in the Jardin des Plantes de Paris, and in 1889, Mollereau (16) reported chorioptic mange on common goats in rural France. Kemper *et al.* (12) observed chorioptic mange on about five per cent of more than 2000 Angora goats in three herds in the western United States. The lesions on the animals sometimes extended beyond the legs to include the abdomen and thorax. Hirst (11) reported the condition from Texas; Gerlach (7) noted it from Germany; and Oudemans (19) recorded it on the legs and muzzle of a goat in Sumatra. Four goat herds from Quebec were examined in the current study. None had lesions, but chorioptic mites occurred on the feet of individuals in three of the herds. One herd, examined more carefully than the others, had six of its 13 animals positive, and three kids born subsequently became infested within a month of birth. From the results of this study it seems possible that chorioptic mites may be more prevalent on goats than is generally maintained, and that mange is an infrequent complication.

*Kindly submitted by J. F. Frank, Animal Pathology Division, Canada Department of Agriculture, Eastern Section Branch Laboratory, Mount Allison University.

(*c*) *Other Hosts**

Chorioptic mites have occurred on mammals other than farm stock. Gerlach (7) stated that Gurlt figured a symbiotic (chorioptic) mite from an elephant, but that no details were available. In 1874, Zürn (29) may have observed chorioptic mites in the ears of a rabbit. These reports are discussed later in this communication. Railliet and Mouquet (24) reported chorioptic mange on the legs, abdomen, and thorax of one of four Barbary sheep (*Ammotragus lervia*) in the London Zoological Gardens. In the same gardens, Hirst (10) noted this malady on the feet of a guanaco (*Lama huanacus*). In the present study, mites—not associated with mange—were collected from both hind and forefeet of two yearling llamas (*Lama huanacus glama*) in the Société Zoologique de Granby (Québec). The observations summarized above indicate that the important sites of infestation of chorioptic mites on both farm and zoo animals are the feet and lower hind legs with some involvement elsewhere. The mites, therefore, display no apparent behavioristic differences on different hosts.

Present Taxonomic Status

Confusion in the literature regarding the scientific names of chorioptic mites persisted until, and sometimes after, the work of Vitzthum in 1943 (27). Vitzthum's comprehensive volume was remarkably complete, but he overlooked recognized species of *Chorioptes*. He also, as well as previous authors (23, 18), erred in some authority citations that were augmented or perpetuated by Radford (22), Baker and Wharton (1), and Sweatman (26). This has necessitated a summary and re-examination of the genus before suggesting its revision.

The chorioptic mite from the cow was first described by Héring in 1845 (9) and named *Sarcoptes bovis*. It had been observed 10 years earlier on that host by Kegelaar (*in* Ref. 7), but he did not describe it. Gerlach coined *Symbiotes* in 1857 (7) for the genus of these mites on cattle (*S. bovis*) and horses (*S. equi*), and referred a mite from an elephant figured by Gurlt to the same genus (*S. elephi*). In 1857–58, Delafond and Bourguignon (5) gave the name *Sarcoptes caprae* to what we now recognize as a chorioptic mite from goats. Realizing that this mite had features of both *Sarcoptes* and *Dermatodectes* (*Psoroptes*), these same authors renamed it *Sarco-Dermatodectes* in 1862 (6). In 1859, however, Gervais and Van Beneden had already adopted *Chorioptes* for this same mite of Delafond and Bourguignon, removing it from *Sarcoptes* in which it had been placed incorrectly, and also rejecting Gerlach's *Symbiotes* as a homonym. The generic name with priority is therefore *Chorioptes*, and the type species is *C. caprae*. At the time of Gervais and Van Beneden, four species were referable to the genus—*C. caprae*, *C. bovis*, *C. equi*, and *C. elphi*. New hosts have been observed subsequently. Morphological differences

*According to Brumpt (2), chorioptic mites were observed by Zürn on the head of a man with alopecia; and Moniez observed them in the nose of another person. These were probably incidental parasites. The present writer has had chorioptic mites crawling repeatedly on his hands and arms for over 2 years with no suggestion of sensitization.

between the mites from the different hosts were absent or slight. *Chorioptes*, however, like *Sarcoptes*, *Psoroptes*, *Otodectes*, and *Demodex*, was thought to be host specific and different physiologically. Therefore, convention dictated adoption of a new specific (or sometimes subspecific) name for the mite as it occurred on each host. There was one exception; that was Hirst's (10) collection of chorioptic mites from the guanaco. He refrained from creating a new species, or even attempting to refer the mites to a recognized species. Two years later, however, Hirst (11) described a second caprine species of *Chorioptes* taken on a domestic goat in Texas. A summary of the species of *Chorioptes*, with their synonyms, is shown below:

Chorioptes Gervais and Van Beneden, 1859

Type: *Sarcoptes caprae* Delafond and Bourguignon, 1857–58.

Synonyms: *Symbiotes* Gerlach, 1857; *Dermatophagus* Fürstenburg, 1861; *Sarco-Dermatodectes* Delafond and Bourguignon, 1862; *Dermatophagus symbiotes* Verheyen, 1862; *Chorioptes symbiotes* Railliet, 1893; *Symbiotes communis* Cave, 1909; *Chorioptes bovis* Railliet and Mouquet, 1919; *Chorioptes communis* McKenna and Pulsford, 1947.

Domestic goat.—*Chorioptes caprae* (Delafond and Bourguignon, 1857–58) Gervais and Van Beneden, 1859.

Synonyms: *Sarcoptes caprae* Delafond and Bourguignon, 1857–58; *Chorioptes symbiotes* var. *caprae* Railliet, 1893; *Chorioptes bovis* var. *caprae* Neveu-Lemaire, 1938.

Domestic goat.—*Chorioptes texanus* Hirst, 1924.

Domestic cow.—*Chorioptes bovis* (Héring, 1845) Gervais and Van Beneden, 1859.

Synonyms: *Sarcoptes bovis* Héring, 1845; *Symbiotes bovis* Gerlach, 1857; *Dermatophagus symbiotes* var. *bovis* Verheyen, 1862; *Chorioptes symbiotes* var. *bovis* Railliet, 1893; *Chorioptes bovis* var. *bovis* Neveu-Lemaire, 1938.

Horse.—*Chorioptes equi* (Gerlach, 1857) Gervais and Van Beneden, 1859.

Synonyms: *Symbiotes equi* Gerlach, 1857; *Symbiotes spathiferus* Mégnin, 1872; *Chorioptes symbiotes* var. *equi* Railliet, 1893; *Chorioptes bovis* var. *equi* Neveu-Lemaire, 1938.

Domestic sheep.—*Chorioptes ovis* (Zürn, 1874) Baker and Wharton, 1952.

Synonyms: *Dermatophagus ovis* Zürn, 1874; *Chorioptes symbiotes* var. *ovis* Railliet, 1893; *Symbiotes communis* var. *ovis* Cave, 1909; *Chorioptes bovis* var. *ovis* Neveu-Lemaire, 1938; *Chorioptes communis* var. *ovis* McKenna and Pulsford, 1947.

Elephant.—*Symbiotes elephi* Gerlach, 1857.

Barbary sheep.—*Chorioptes bovis* var. *ammotragi* Railliet and Mouquet, 1919.

Domestic rabbit.—*Chorioptes symbiotes* var. *cuniculi* Railliet, 1893.

Synonyms: *Chorioptes bovis* var. *cuniculi* Neveu-Lemaire, 1938; *Chorioptes cuniculi* Vitzthum, 1943.

Comparative Biology and Morphology

Since it was convention, rather than experimental evidence, that persuaded most observers to name each chorioptic mite differently as it was discovered on a new host, it was essential in the current study to ascertain the validity of the accepted species and their host specificity. This problem has been approached using six different methods, each summarized below under its own subheading. Mites for the experiments were from five hosts—cow, horse, goat, sheep, and llama—and, for the first four, presumably represented the species of *Chorioptes* (*C. bovis*, *C. equi*, *C. caprae*, and *C. ovis*) associated with that host. The hosts were from Quebec, but came from widely separated premises.

1. Life History and In vitro Survival

It has been shown already that the feet and lower hind legs are the important sites of infestation on all hosts. The life cycle with seven motile stages and the habits of the mites from each of five hosts have been observed *in vitro* to be identical for each. The period required for completion of the cycle was also similar, and varied within the range (18 to 28 days) established previously for *C. bovis* from cattle. Not only was it possible to culture the mites on epidermic debris from their own host, but also on material from the feet of each of the other four hosts. Table V shows that all cycles in repeated trials were completed in the standard period, and there was no apparent loss of vigor or any indication of sterility in the second and third generations. These observations proved that epidermal material from each host contained the necessary nutrients for culturing mites taken from each of the other hosts.

2. Cross-breeding

The ultimate criterion of a species is usually its reproductive isolation from other species. Hence, experiments were conducted to permit cross-breeding of mites from the different hosts. The viability of any progeny would indicate success since it was demonstrated in the life history that eggs are not laid

TABLE V

PERIOD (IN DAYS) FOR LIFE CYCLE ON EPIDERMIC DEBRIS FROM EACH OF FIVE HOSTS

	Debris source				
Egg source	Cow	Horse*	Goat	Sheep‡	Llama
Cow		21–25	19–25	22–27	25–27
Horse*	18–26		18–23	25–27	26
Goat	18–21	23		26	27
Sheep†	19	21	20		26
Llama	21	23	19	26	

* Clydesdale or Belgian.
† Suffolk.
‡ Suffolk, Leicester, North Country Cheviot, or Hampshire Down.

parthenogenetically. About a dozen motile pubescent females or quiescent female protonymphs from one host were placed in a vial with adult males from a different host. In all instances, the epidermal material in the vial was from either cattle or horses, and had been sterilized previously by heat to kill any mites or eggs that might have been present. Males and females from each of the five hosts (18 of a possible 20 combinations used) did copulate and the females laid eggs which in turn produced fertile progeny and eggs in the second generation. Mites from the five sources, therefore, were compatible reproductively.

3. Comparative Survival on Foreign Epidermal Material

The hosts found naturally infested with chorioptic mites are from three families of hoofed mammals—Bovidae, Equidae, and Camelidae. If the mites from the different hosts are truly different, then it is conceivable that mites from one member of a family might respond differently on epidermal material from another member of that same family than would mites from members of each of the other two families. To determine this, eggs from the cattle, horse, and llama cultures were transferred to vials containing epidermic debris from other members of their families. Table VI shows that the results were similar on three species of oxen, two equids, and three camelids regardless of the original host of the mites. This emphasizes further the similarity of the mites from the different hosts.

4. Comparative Survival within the Family Bovidae

The next approach to the problem of host specificity was similar to the last one. It was to use epidermic debris from antelopes, since these in the family Bovidae link the oxen on the one hand to the sheep and goats on the other.

TABLE VI

COMPARATIVE SURVIVAL OF MITES ON EPIDERMIC DEBRIS FROM CLOSE RELATIVES

Debris source	Egg source		
	Cow	Horse	Llama
Zebu	C	C	C
Indian water buffalo	C	C	C
American bison	P	P	P
Grant's zebra	C	C	C
Donkey No. 1	C	C	C
Donkey No. 2	P	P	P
Arabian camel	D	D	D
Bactrian camel	D	D	D
Guanaco	D	D	D

C: Life cycle completed in the usual 3-week period.
P: Prolongation of early developmental stages with only the occasional, if any, mite reaching maturity.
D: Death from apparent starvation in the larval stage following the hatching of the eggs.

Eggs from cow cultures as well as the goat or sheep cultures were placed in vials with epidermal material from the eland (*Taurotragus oryx*), Bohor reedbuck (*Cervicapra bohor*), and white-bearded gnu (*Connochaetes albojubatus*). The results were similar regardless of the source. The life cycle was completed on material from the eland, but reached only the nymphal stages on material from the two other antelopes. No differences, therefore, were detected between the mites from cattle and those from goats or sheep.

5. Induced Natural Transmission

The preceding laboratory studies indicated the non-specificity of chorioptic mites, but this required confirmation by transmission of the mites from one host species to another. Four individual experiments were conducted. Two uninfested goats were placed with a heifer having a general infestation; one calf, raised on milk from a pail and never in association with its dam or other cattle, was maintained with a positive goat herd; and a similar calf was corraled with three positive Suffolk sheep. Two protonymphal mites were located on the muzzle of one of the two goats with the positive heifer 1 week following their first association. This goat had a habit of rubbing its head against the heifer, suggesting that the mites were transferred by direct bodily contact. Within 3 weeks of the initiation of the experiments, both goats and the two calves had reproducing mite populations on at least two of their feet. This proves that the mites are capable of transfer between, and reproduction on, different host species of hoofed mammals.

6. Morphological Comparisons

Regardless of the preceding data, it is theoretically possible that more than one species was involved in the experiments, both or all of which showed non-specificity and which might interbreed and produce fertile progeny under unnatural conditions as do black and mallard ducks or the gayal and zebu. It is possible also that the mites might be genetically the same, but respond differently in different environments (hosts), thus displaying ecophenotypic variation. It was necessary therefore to make morphological comparisons of the mites on the same and different hosts, as well as with descriptions of previous workers. A summary of the latter will be used to introduce this section. In 1857, Gerlach (7) made rather imperfect drawings of the adult chorioptic mites, declaring the mite on the horse and that on the cow to be host specific and therefore distinct (*C. equi* and *C. bovis*), although morphologically indistinguishable. In 1872, Mégnin (14) redescribed the mite from the horse, and made more detailed drawings than Gerlach of the adult mites. Additionally, he pointed out some discrepancies between Gerlach's figures and what he saw. Mégnin also recognized the presence of some immature stages, although only a meager attempt was made to describe them. On the differences noted on the adult mites, Mégnin created a new species, (*C.*) *spathiferus*, but stated that he would not be surprised if it were really the same as *C. equi*. Five years later (15) he reduced the original *C. equi* to a synonym with his new name. With this arrangement, he was willing, apparently, to

overlook the slight morphological differences he had noted originally. About 50 years later, Oudemans (19) recognized the inadequacies in the original drawings of *C. caprae* by Delafond and Bourguignon (5), so he redescribed this species from material sent him from a goat in Sumatra. Oudemans described and figured both the immature and adult stages. He then noted the differences between his specimens and the drawings and notes on *C. equi* by Mégnin (14). In 1947, Palimpsestov (20) recognized the presence of the immature and quiescent stages of *C. equi* from horses in Russia. The number of stages seen by him for *C. equi* were the same as those noted by Oudemans for *C. caprae*. All previous authors had relied on the drawings of their predecessors for purposes of comparison, rather than examining actual specimens. They were obliged, therefore, to accept any descriptive differences as specific differences. The present writer was unable to obtain the actual specimens used by others, and, *in lieu* of this, collected chorioptic mites from as many hosts as possible and accepted each as being representative of the species generally attributed to that host. Suffice to say that all morphological characters, including the positions of the setae, etc., were identical on each of the seven motile stages from each of five hosts (cow, horse, goat, sheep, and llama). A series of critical measurements were also made to ascertain the presence or absence of any ecophenotypic variation. These included a comparison of measurements on 10 to 15 individuals from each host of the length and width of the dorsum, length of the propodosomal plate, each leg, caruncles I or II, and seven setae (lateral propodosomal, large dorsal, propodosomal plate, lateral metapodosomal, opisthosomal, ventral propodosomal, and femoral II). Any measurements given by previous authors were compared also. These data have been deposited in the library of the Institute of Parasitology, Macdonald College. They show that variations occur for the same structure on different individuals from each host, but all fall within the same approximate range. No differences were therefore apparent.

A comparison will now be made with some of the figures and comments of previous authors. The setae on the opisthosomal lobes of the adult male on *C. equi* are from independent setal bases in the drawings of Gerlach (7). Mégnin (14) first showed the correct groupings of the setae in common bases as shown here in Fig. 33. Oudemans (19) also showed this arrangement for *C. caprae*. Oudemans pointed out differences in the shape of the propodosomal plate of Mégnin's drawing of *C. equi* and his own *C. caprae*. These differences are, however, referable to individual variation as shown here in Figs. 4 to 11. As Oudemans stated, the long dorsal propodosomal setae as drawn by Mégnin appear directly behind the short setae near the posterior corners of the propodosomal plate, whereas on Oudeman's specimens they were posterolateral to the plate. The actual positions of setae in general vary considerably, and it is not impossible that Mégnin actually saw what he drew, although neither Oudemans nor the present writer have seen the pattern Mégnin described. Oudemans indicated that the sides of the vulva in *C. caprae* are straight, and not in the artistic shape of an inverted lyre as shown for *C. equi* by Mégnin. The present author agrees with Oudemans. Mégnin shows the three pairs

of setae at the posterior end of the vulva all lateral to the opening of the vulva. Oudemans, for *C. caprae*, and the present writer for all species, show only two pairs of the setae lateral to the slit of the vulva, and the third pair medial to the vulva opening. On the same ovigerous female, Oudemans shows two pairs of anal setae. The present writer has found one pair of these to be adanal rather than anal, and this is frequently displaced some distance laterally. Judging from his drawings, Oudemans overlooked setae on the dorsum on all stages. He shows, for example, eight pairs of idiosomal setae on the pubescent female, whereas the present writer has 10 pairs; the male deutonymph of Oudemans has four pairs of ventral metapodosomal setae, but this author has five pairs. A number of other examples are possible, but seem unnecessary.

Zürn (28), when he described *C. ovis* from a sheep, simply stated that it resembled exactly the chorioptic mite from the horse, except for a possible difference in size. The size differences he observed fall within the range of measurements noted in this study for a number of individuals. The above data suggest that during the past 100 years there has been a gradual increase in the detail on the drawings of the mites concomitant with improved microscopic equipment. The present writer is of the opinion, therefore, that differences between drawings of the mites of the various authors are not real.

In summary, there are no morphological differences between the four species of *Chorioptes* from domestic hosts (*C. bovis*, *C. equi*, *C. caprae*, and *C. ovis*), as well as those mites from a llama. This fact, together with their identical life cycles, capacity to interbreed and transmit to at least some other hosts, and their *in vitro* survival on epidermal material from each other and some foreign material, demonstrates to this writer that perpetuation of the four species is untenable. It is believed that only one of the above species exists, which infests each of the hosts, and the name with priority is *C. bovis*. The four remaining specific or subspecific names in the genus will be discussed following the next section of this report.

III. Mite Survival on Epidermic Debris from Various Animals

The synonymy of chorioptic mites that occur naturally on a variety of domestic hosts suggested that some measure be made of their degree of potential ubiquitousness. The experiment was conducted by obtaining hair with epidermic debris from various ungulates within the familes Camelidae, Bovidae, and Equidae, in which natural hosts occur, together with epidermal material from members of the related family Cervidae.

Approximately 200 eggs were collected from the cattle culture vials and placed in an unused vial with epidermal material from the mammal to be tested. Determination was made of the time required for the development of the various stages in the life cycle of the mite. The vials were examined every second or third day. Table VII summarizes the results. Observations were generally made in duplicate, but results that were virtually the same (as they often were) are not repeated in the table. Members of Bovidae are arranged in the table according to the recent classification of Pilgrim (21).

TABLE VII

In vitro SURVIVAL OF MITES EMERGENT FROM EGGS ON EPIDERMAL MATERIAL FROM VARIOUS MAMMALS

Animal	Individual No.	Site	Period, days: Eggs and larvae	Nymphs†	Adult males	Attachment pairs	Ovigerous females	Whole cycle	Summary*
Order Artiodactyla									
Family Camelidae (see Tables V and VI)									
Family Cervidae									
Reindeer, *Rangifer tarandus*	1	Hock	1–9	5–16	11	11	19	21	C♀
	2	Hock	1–32	17–32	23	N.P.*			P
Wapiti, *Cervus canadensis*	1	Side	1–14	9–25	17	17	23	26	C♂
	1	Shoulder	1–18	10–35	22	22	31–45	N.P.	P♂
	2	Back	1–5	N.P.					D
White-tailed deer, *Odocoileus virginianus*	1	Feet	1–9	9–23	16	16	24	26	C♂
	2	Front feet	1–7	5–17	14	14	17	19	C♀
	2	Hind Feet	1–12	7–36	N.P.				P♂
	2	Tail base	1–13	11–18	N.P.				P♂
	3	Feet	1–12	9–34	N.P.				P♂
	3	Feet	1–14	12–28	N.P.				P♂
	4	Side	1–31	20–48	36	36	N.P.		P♂
	4	Side	1–23	12–42	29	N.P.			P♂
	4	Side	1–25	13–43	N.P.				P♂
	5	–	1–11	N.P.					P♂
(fawn)	6	Feet	1–15	13–18	N.P.				P♂
(fawn)	7	Feet	1–20	7	N.P.				P♂
(fawn)	8	Feet	1–19	8–22	N.P.				P
Mule deer, *Odocoileus hemionus*	1	Side	1–16	14–21	N.P.				P♂
	1	Side	1–6	6–10	N.P.				P♀
Fallow deer, *Dama vulgaris* (fawn)	1	Feet	1–12	12–15	N.P.				P
(fawn)	1	Feet	1–9	N.P.					P
(fawn)	2	Feet	1–21	N.P.					P
Moose, *Alces americana*	1	Shoulder	1–31	11–50	31	31	N.P.		P
	1	Shoulder	1–32	15–32	N.P.				P
	1	Shoulder	1–16	16–42	N.P.				P
	2	Side	1–4	N.P.					D
Family Bovidae									
Antilocaproidea									
Antilocaprinae									
Pronghorn, *Antilocapra americana* (kid)	1	Feet	1–11	9–25	N.P.				P
(kid)	1	Feet	1–12	12–19	N.P.				P
(kid)	2	Feet	1–16	8–23	N.P.				P
(kid)	2	Feet	1–9	9–24	N.P.				P

TABLE VII—*Concluded*

In vitro SURVIVAL OF MITES EMERGENT FROM EGGS ON EPIDERMAL MATERIAL FROM VARIOUS MAMMALS—*Concluded*

Animal	Individual No.	Site	Period, days: Eggs and larvae	Nymphs†	Adult males	Attachment pairs	Ovigerous females	Whole cycle	Summary*
Aegodontia									
Caprinae									
Domestic goat, *Capra hircus* (see Tables V and VIII)									
Asiatic ibex, *Capra sibirica*	1	–	1–14	12–18	18	N.P.			P
	1	–	1–8	N.P.					P
Markhor, *Capra falconeri*	1	Shoulder	1–7	N.P.					D
Domestic sheep, *Ovis vignei* × *O. musimon* (see Tables V and VIII)									
European mouflon, *Ovis musimon*	1	–	1–20	N.P.					P
	2	–	1–5	N.P.					D
Bighorn, *Ovis canadensis*	1	Side	1–20	N.P.					P
	2	–	1–5	N.P.					D
Barbary, *Ammotragus lervia*	1	Side	1–15	N.P.					P
	1	Side	1–7	N.P.					D
Saiga, *Saiga tatarica*	1	–	1–9	8–10	N.P.				P
	1	–	1–16	15–17	N.P.				P
	2	–	1–19	14–21	N.P.				P
	3	–	1–19	N.P.					P
Ovibovinae									
Musk ox, *Ovibos moschatus*	1	–	1–7	N.P.					D
Neotraginae									
No representative									
Gazellinae									
Impala, *Aepyceros melampus*	1	–	1–11	7–28	19	19	29	32	P
	1	–	1–9	7–28	17	19	24	27	C
Blackbuck, *Antilope cervicapra*	1	–	1–7	7–19	N.P.				P
	1	–	1–15	N.P.					P
No name									
Alcelaphinae									
White-bearded gnu, *Connochaetes albojubatus*	1	–	1–14	8	N.P.				P
	1	–	1–11	N.P.					P
Hippotraginae									
No representative									
Reduncinae									
Bohor, *Cervicapra bohor*	1	–	1–21	7–51	N.P.				P
	1	–	1–28	12–23	N.P				P

Cephalophinae									
Yellow-backed duiker,	1	–	1–26	15–26	N.P.				P
Cephalophus sylvicultrix	1	–	1–16	14–16	N.P.				P
Tragelaphoidea									
Tragelaphinae									
Nyala, *Tragelaphus angasi*	1	–	1–7	7–20	14	14	17	20	C
	2	–	1–16	8–21	16	16	17	21	C
	3	–	1–8	5–43	19–38	21–35	N.P.		P
Harnessed antelope,	1	–	1–10	7–16	14	14	35	N.P.	P
Tragelaphus scriptus	1	–	1–12	9–44	26	N.P.			P
Delamere's bushbuck, *Tragelaphus delamerei*	1	–	1–18	9–25	N.P.				P
Eland, *Taurotragus oryx*	1	–	1–11	7–27	16	18	23	27	C
Boselaphinae									
Nilgai, *Boselaphus tragocamelus*	1	–	1–9	9–31	31	N.P.			P
	1	–	1–15	N.P.					P
Bovinae									
Indian water buffalo, *Bubalus bubalis*	1	Buttocks	1–8	8–32	16	16	18	20	C
African buffalo, *Syncerus caffer*	1	–	1–20	15–34	N.P.				P
	1	–	1–22	N.P.					P
Yak, *Poephagus grunniens*	1	–	1–10	N.P.					P
	2	–	1–4	N.P.					D
Wisent, *Bison bonasus*	1	–	1–4	N.P.					D
American bison, prairie race,	1	Shoulder	1–11	9–14	21	21	N.P.		P
Bison bison typicus	2	–	1–6	N.P.					D
Gaur, *Bibos gaurus*	1	–	1–4	N.P.					D
Domestic cow, *Bos taurus* (see Tables V and VIII)									
Zebu, *Bos indicus*	1	Feet	1–11	9–19	17	17	22	27	C
	2	Feet	1–19	7–31	17	17	24	N.P.	P
Order Perissodactyla									
Family Equidae									
Domestic horse, *Equus caballus* (see Tables V and VIII)									
Donkey, *Equus africanus*	1	Feet	1–9	9–28	19	19	24	28	P
	2	Feet	1–7	7–21	15	18	21	23	C
	3	Feet	1–14	9–20	N.P.				P
	4	Feet	1–15	15–32	N.P.				P
Grant's zebra, *Equus burchelli granti*	1	Buttocks	1–10	7–18	15	15	23	25	C
Chapman's zebra, *Equus burchelli chapmani*	1	Shoulder	1–11	8–28	28	N.P.			P
	1	Shoulder	1–24	10–37	N.P.				P

* N.P.: Not produced. When N.P. occurs under "Whole cycle", it denotes that all stages developed, but that the ovigerous females failed to produce eggs.
C: Life cycle completed in the usual 3-week period.
P: Prolongation of early developmental stages with only the occasional, if any, mite reaching maturity.
D: Death from apparent starvation in the larval stage following the hatching of the eggs.

† May include one or more nymphal stages.

The pronghorn is now included in this family rather than in Antilocapridae, and the saiga is in the goat and sheep subfamily (Caprinae) rather than with the "antelopes". It will be noted also that the musk ox is in a subfamily more closely related to the sheep and goats than to the oxen. Hair was obtained from at least one representative in 10 of the 12 subfamilies within Bovidae, as well as from three species of Camelidae, six of Cervidae, and three species of Equidae, one of which has two subspecies.

The results show that *C. bovis* can survive *in vitro* to at least some extent on epidermal material from many ungulates. The life cycle was completed in the usual 3-week period on hair from some, but not all, individuals of reindeer, wapiti, and white-tailed deer in Cervidae; on debris from three species of antelopes (impala, nyala, and eland); on material from the Indian water buffalo and zebu among the oxen; as well as on epidermic debris from the donkey and Grant's zebra. Mites on most material tested, however, showed prolongation of the early developmental stages and premature death. Prolongation was sometimes marked. Compare the standard survival period of 3 to 5 days for larvae on Holstein–Friesian material with the persistance of some larvae up to 31 days on material from one white-tailed deer, or up to 32 days on debris from a moose (Table VII). Sometimes nymphal prolongation was marked also on material from some members of the two species above (from 20 to 48 days on white-tailed deer and from 11 to 50 days on moose), as well as on one nyala (5 to 43 days), a harnessed antelope (9 to 44 days), and a Chapman's zebra (10 to 37 days). As perhaps already implied, results varied between wide limits on material from different individuals of the same species. Hair was from diverse, and sometimes unknown, body areas, but the significance of this, or the age of the mammal, on the survival of the mites is unknown. The complete life cycle observed on material from one wapiti was in contrast with results on material from another where no survival whatever could be induced. Material from one bighorn sheep, a European mouflon, and a yak supported larvae for a prolonged period, while fairly rapid larval death occurred on material from another individual of each species. Early larval death was observed also on material from one of two fallow deer, one of two moose, one Markhor goat, one musk ox, one gaur, and one wisent. Like larvae on material from the European bison, some died rapidly on epidermal material from one American bison, but some mites developed right up to attachment pairs on debris from another. These data suggest the presence of marked differences between individuals in their capacity to support *C. bovis*, with little precise reference to the actual species tested. The experiment also indicates no trend concordant with the phylogenetic relation between the possible hosts. It seems possible, too that sylvatic infestations may occur, although natural infestations have been observed only on animals in captivity.

The individual variation noted for wild ungulates may be more consistent within a species or perhaps a breed of domestic stock. It is also true that an unsusceptible breed would not require treatment in a farm eradication program. To determine the susceptibility of various farm animals, hair with

epidermic debris was collected from 45 breeds of cattle, goats, sheep, and horses of purebred, registered stock. Table VIII shows that the life cycle was completed *in vitro* in the usual 3-week period on epidermal material from 12 of 14 cattle breeds, but showed developmental prolongation or early death on material from members of the two remaining breeds. Results also showed individual variation within a breed among Jersey, Guernsey, and Brown Swiss cattle, and such might have been more general on other breeds if larger numbers of individuals had been sampled. No relation existed between mite survival and breeding of the animal mainly for milk or meat production. A fair abstraction would be that most cattle breeds, but not all individuals within those breeds, appear to be susceptible to *C. bovis*.

Fewer life cycles were completed on debris from breeds of goats, sheep, and horses (Table VIII) than on material from cattle. The observations on material from sheep make it convenient to divide the breeds of sheep into three groups:

(*a*) "Bare-legged" breeds—those with hair, rather than wool, on the lower legs and head. Principally, these are the mutton breeds.

(*b*) Breeds with hair confined to the feet, having wool on the body and legs above the pastern. Heads only partly covered by wool.

(*c*) Breeds essentially covered with wool.

The experiments were purposely biased in favor of the first group, and to a lesser extent the second, because of preliminary *in vitro* success on hair from the feet of Suffolk sheep compared with utter failure on wool from their bodies. Mites in the wool adhered to the globules of lanolin, preventing much of their motility and apparently causing their death by gradual starvation. Similar results occurred on material from the feet of the Shropshire and Southdown (Table VIII) in group (*c*). This was not true, however, on material from the Rambouillet, where prolongation of the nymphal stages occurred, nor does it have an absolute relation with natural infestations, some of which have occurred on the scrota (where there is generally much lanolin) of Negretti and Corriedale rams (see Natural Infestations). The explanation may lie in an increased tackiness of the lanolin under *in vitro* conditions at 35° C., a temperature that is probably a little higher than that in the wool on the host.

Complete development occurred *in vitro* on epidermal material from the feet of an individual in each breed of Suffolk, Leicester, South Country Cheviot, and Lincoln, but premature death occurred on material from other individuals from the first three of these breeds. The complete cycle occurred on material from only one individual (a Hampshire Down) in group (*b*), and failed to produce mature mites on material from four other individuals among the three breeds in this group. These data suggest considerable variation in susceptibility between individuals irrespective of breed insofar as groups (*a*) and (*b*) are concerned, and together with what is known on natural infestations, indicate that sheep are less suitable hosts for *C. bovis* than domestic cattle.

TABLE VIII

In vitro SURVIVAL OF MITES EMERGENT FROM EGGS ON EPIDERMAL MATERIAL FROM DIFFERENT BREEDS OF CATTLE, GOATS, SHEEP, AND HORSES

Animal	No. of individuals	Site	Period, days: Eggs and larvae	Nymphs	Adult males	Attachment pairs	Ovigerous females	Whole cycle	Summary*
Domestic cattle									
Holstein–Friesian	10	Feet	–	–	–	–	–	16–27	C
Ayrshire	4	"	–	–	–	–	–	18–24	C
French Canadian	3	"	–	–	–	–	–	16–18	C
Jersey	2	"	–	–	–	–	–	22–27	C
	1	"	1–8	6–18	16	16	N.P.*		I
	2	"	1–8	6–20	N.P.				I
Guernsey	1	"	–	–	–	–	–	19	C
	2	"	1–14	7–14	N.P.				P
	1	"	1–14	12–37	N.P.				P
	1	"	1–7	N.P.					I
Brown Swiss	3	"	–	–	–	–	–	18–20	C
	1	"	1–14	N.P.					P
Red Polled	2	"	1–6	N.P.					D
Charallais†	1	"	1–5	N.P.					D
Hereford	1	"	–	–	–	–	–	21	C
Shorthorn	2	"	–	–	–	–	–	18–20	C
Aberdeen Angus	4	"	–	–	–	–	–	19–27	C
Belted Galloway	1	Buttocks	1–13	9–26	16	16	N.P.		P
	1	Buttocks	1–11	9–21	16	17	19	21	C
Devon	3	Feet	–	–	–	–	–	19–23	C
Scotch Highland	2	"	–	–	–	–	–	21–23	C
Goats									
Toggenburg	3	Feet	–	–	–	–	–	19–22	C
Nubian	1	"	–	–	–	–	–	22	C
	2	"	1–9	N.P.					P
Saanen	1	"	1–8	N.P.					P
Sheep									
Group (*a*)									
Suffolk	2	Feet	–	–	–	–	–	25–27	C
	1	"	1–19	15–39	27	25	N.P.		P
	1	"	1–10	10–31	15	15	N.P.		P
	2	Muzzle	1–21	16–23	N.P.				P
	6	Feet	1–12	N.P.					P
North Country Cheviot	2	"	1–20	9–26	N.P.				P
	1	"	1–22	6–29	19	19	22	N.P.	P
South Country Cheviot	2	"	–	–	–	–	–	25–27	C
	1	"	1–17	9–30	17	17	N.P.		P
	1	"	1–15	9–32	N.P.				P

Leicester	1	"	–	–	–	–	–	26	C
	3	"	1–11	7–35	15–20	15–27	28–34	N.P.	P
	2	"	1–9	9–30	17–34	N.P.			P
	1	"	1–16	N.P.					P
Lincoln	1	"	–	–	–	–	–	21	C
Cotswald	1	"	1–6	N.P.					D
Ryeland†	1	"	1–15	N.P.					P
Group (*b*)									
Hampshire Down	1	Feet	–	–	–	–	–	22	C
	2	"	1–11	7–23	16–23	N.P.			P
Oxford Down	2	"	1–15	11–30	25–28	27	N.P.		P
Dorset Horn	1	"	1–21	18–24	N.P.				P
	1	Muzzle	1–16	14–28	N.P.				P
Group (*c*)									
Rambouillet	2	Feet	1–14	12–21	N.P.				P
Shropshire	1	"	1–7	N.P.					P
Southdown	2	"	1–5	N.P.					D
Horses									
Clydesdale	3	Feet	–	–	–	–	–	21–25	C
Belgian	2	"	–	–	–	–	–	22–25	C
Percheron	1	"	1–16	12–23	N.P.				P
Suffolk	1	"	1–14	9–29	21	21	N.P.		P
French Canadian	1	"	1–24	19–28	23–32	N.P.			P
	2	"	1–15	9–23	N.P.				P
Palomino	1	"	1–24	14–24	N.P.				P
Arabian	1	"	1–16	7–22	19	19	28	31	P
	2	"	1–17	8–27	N.P.				P
Hunter	2	"	1–13	9–29	N.P.				P
	1	"	1–11	9–25	18	18	N.P.		P
American saddle	1	"	1–18	16–20	N.P.				P
Thoroughbred	1	"	1–16	11–28	28	28	35	N.P.	P
	1	"	1–17	11–31	20	23	N.P.		P
Standard-bred	1	"	1–18	9–25	N.P.				P
French Coach	1	"	1–7	7–31	16	16	24	27	C
Tennessee Walking	1	"	1–19	17–37	27–39	N.P.			P
Hackney	2	"	1–28	8–25	N.P.				P
Shetland pony	2	"	1–16	9–25	25–27	N.P.			P

* See Table VII for explanation.
† Sample too small for a truly satisfactory trial.

Material from 15 horse breeds was tested (Table VIII). *C. bovis* completed its cycle on only four of these, two of which were draft breeds (Clydesdale and Belgian). The third breed, a French Coach, is rare in Canada, and mites on material from the fourth breed (Arabian) showed prolonged development on three individuals but completed the cycle on one of these even though it took 31 days.

Taken as a whole, the different breeds of cattle appear to be more suitable for the survival of *C. bovis* than the breeds of sheep and horses. Too few goats of purebred stock were available to justify a generalization regarding them, but the scant results listed in Table VIII and the fact that the life cycle was completed on material from all six individuals tested that were of mixed breeding, together with the natural infestations, suggest that goats may be more suitable hosts than sheep or horses. It seems likely from the results on material from both wild and domestic ungulates that the domestic cow is the most natural host species, and is probably the host primarily responsible for the current cosmopolitan distribution of the parasite.

Some survival of *C. bovis* has occurred on epidermal material from a few unrelated animals. Prolongation of the larval and nymphal stages (up to 18 days) occurred on epidermic debris from three guinea pigs. On material from a fourth, one ovigerous female developed, lived 6 days and produced two eggs, completing the life cycle in 32 days. Observations on material from domestic rabbits are included elsewhere in this report. No growth occurred on epidermic debris from man, swine, dog, cat, little brown bat (*Myotis lucifugus*), bumblebee (*Bombus* sp.), or housefly (*Musca domestica*). Nor did mites survive on debris from the bodies of domestic chickens, but larvae lived for prolonged periods up to 19 days on material from the shank of three breeds (barred Plymouth Rock, Rhode Island Red, buff Orpington) as well as for periods up to 17 days on similar material from a black duck (*Anas rubripes*), ruffed grouse (*Bonasa umbellus*), and domestic turkey. On the last host, the larvae transformed into protonymphs that lived up to 15 days. Clearly some of the nutritional requirements of *C. bovis* appear to occur on various animals.

Most of the foregoing experiments were carried out *in vitro*. It is desirable therefore to estimate the relation between the *in vitro* culture method with possible or real natural infestations. For cattle there was a direct relation. Mites were readily cultured at 35° C. and 80% R.H. on epidermic debris and hair from various cattle breeds with natural infestations. This was also true for goats of mixed breeding, and for Clydesdale and Belgian horses. However, a Percheron horse was found with a few mites in all stages of development on its pasterns, but epidermal material from the same sites would not support survival *in vitro* beyond that of nymphs (Table VIII). Prolongation of the larval and nymphal stages was observed in this instance. Similar prolongation and premature death occurred *in vitro* on epidermal material from six of eight Suffolk sheep, although each was infested naturally. The life cycle was completed *in vitro* on material from the two other Suffolk

sheep. These results suggest that the *in vitro* culture method is probably not perfectly suitable for the mites. This is also borne out since the mites, although they can be maintained for many months by this method, could not, it would seem, be perpetuated *in vitro* indefinitely. These results in turn suggest that the *in vitro* prolongation that was observed on material from some of the cervids, bovids, and equids could possibly reflect potential natural infestations in nature in addition to those individuals which provided epidermal material suitable for completion of the mite's life cycle. The prolongation itself on epidermal material from different sources in a constant environment suggests that feeding, or some factor related thereto, influences the period required for completion of a stage in the life cycle of the mite. It would seem that much of the variation even on epidermal material from the domestic cow is attributable to differences in feeding.

Because of the suitability of epidermic debris from a variety of mammals in different families and even orders for the *in vitro* rearing of *C. bovis*, it is impossible to conjecture on a possible geological age of the parasite from its hostal relations. No sylvatic infestations of *C. bovis* have been observed, but as more mammals become domesticated and important agriculturally, chorioptic mites may acquire veterinary significance on additional ungulates. The experimental results suggest that potential animals in this category are the reindeer in Lapland and the northwestern parts of North America, deer in British Columbia and elsewhere, llamas in South America, zebus and Indian water buffaloes in Asia, and the eland in Africa. The geographical location of the hosts, or their habits may, however, influence the size of a potential mite population.

It is now necessary to return to the taxonomic problem that underlies this communication. Since variation in susceptibility is referable to the individual rather than the species of ungulate, it seems to the present writer that it would be more realistic to regard the *in vitro* early death of *C. bovis* on material from a Barbary sheep (Table VII), in contrast with the natural infestation observed on another individual of this species and named *C. bovis ammotragi* by Railliet and Mouquet (24), as an example of individual differences in susceptibility rather than maintaining that the mite is a distinct parasite. The same argument is applicable to the *in vitro* results on material from a guanaco (Table VI) and the natural infestation observed on this host by Hirst (10). Two other reports require special comment. Apparently Gurlt (*in* Ref. 7) observed a chorioptic mite from an elephant, although no details were recorded. Elephants become infested with the burrowing sarcoptic mite, but it is difficult to imagine how an elephant's hide would be suitable for maintaining a surface-feeding mite. Adult elephants are, of course, virtually hairless. An immature Indian elephant (*Elephas maximus*) with some hair was scraped in the present study for the purpose of collecting hair and debris for attempts to rear chorioptic mites *in vitro*. No scurf, however, could be collected, making it impossible to conduct the experiment.

In 1874, Zürn (29) found canker mites in a rabbit's ear of which he said ". . . ich nun nicht als Dermatophagen (Chorioptes) ansprechen konnte, sondern die zur Gattung Dermatokoptes (Psoroptes) gehörten . . .". Since this was in an addendum to a paper, the author may have written it in haste and in so doing inadvertently interchanged *Dermatophagus* (*Chorioptes*) and *Dermatokoptes* (*Psoroptes*) when attempting to report dermatophagic (chorioptic) mites for the first time from the rabbit. Zürn did not give a specific name to the new mite, although Railliet (23) and other subsequent authors gave him credit for naming it *cuniculi*. The matter is confused further, since Zürn went on to state that the new mite was not essentially different from *Dermatokoptes communis* (*Psoroptes communis*), the common ear mite of rabbits. Additional error is possible since the ear canker mite of carnivores (dog, cat, fox, and ferret), which we know now as *Otodectes cynotis*, was at the time of Zürn considered to be a dermatophagic (chorioptic) mite. Therefore, it is at least possible that Zürn, according to the current classification, had observed otodectic and not chorioptic mites. Experimental evidence in the present study shed no light on a possible truism. Otodectic mites from the ear of a dog were maintained *in vitro* with only moderate success using the same method employed for *Chorioptes*, except that the hair and debris were from the inner ear surface of the infested dog. Eggs from this culture were placed with hair and debris taken from inside the pinnae of four rabbits, but the larvae that emerged died from apparent starvation within 5 days. On the other hand, some chorioptic mites from the cow completed their life cycle *in vitro* in 27 days on rabbit ear debris—but only on the 21st trial, the other trials having shown prolonged development of the larval and nymphal stages (up to 30 days)—and died before reaching maturity. On the basis of the original report and experimental data, it seems possible that Zürn did observe chorioptic mites in the ear of a rabbit, but it might be best to hold this record—as well as Gurlt's report of chorioptic mites on an elephant—in abeyance until additional observations are made from these hosts.

This author is of the opinion that the chorioptic mites reported by others from the guanaco, Barbary sheep, and possibly the rabbit and elephant, and, with one exception, given a distinct name, are really *C. bovis*. Another species, *C. texanus*, described by Hirst (11) from a domestic goat in Texas is maintained as being valid. Hirst's description was sufficiently short for the significant section in it to be repeated here *in toto*. He wrote: "male with the flattened blade-like hairs on the abdominal (opisthosomal) lobes very much narrower than in (*Chorioptes bovis*). Outermost hair on each lobe also very different, being quite short and fine, whereas in (*C. bovis*) this hair is very long and thickened basally." Dr. G. Owen Evans of the British Museum (Natural History) kindly confirmed this description from a single mite in the museum's collection. This is the only specimen known to exist. Hirst, however, examined "a few specimens of both sexes . . .", ruling out the possibility of an anomaly, a phenomenon which does sometimes occur in this genus (Fig. 28). Measurements were given as 260 μ long (with the

gnathosoma) and 185 μ wide for the adult male. The ovigerous female measured 298 μ by 205 μ, and was presumably morphologically identical with that of *C. bovis*. Clearly two species of *Chorioptes* are being recognized at present—*C. bovis* (Héring, 1845) Gervais and Van Beneden, 1859* and *C. texanus* Hirst, 1924.

Summary

Chorioptic mange mites have been reared *in vitro* through sequential generations for some months on epidermic debris from cattle under conditions of complete darkness at 35° C. and 80% R.H. A single cycle of the mite consists of an egg, larva, protonymph, deutonymph, and adult. (The last two stages in the female cycle are called the pubescent female and ovigerous female respectively.) Each stage is described. Each stage other than the egg and larva shows sexual dimorphism. Under the *in vitro* conditions, all but one of the stages usually persists from 3 to 5 days. The pubescent female, however, generally persists for 7 or 8 days. All motile stages feed actively. There is an obligatory quiescence between each motile stage which lasts about 1 day. The complete life cycle encompasses about 3 weeks, and the longevity of egg-laying ovigerous females may also be as long as 3 weeks. Non-laying females and adult males live up to 7 or 8 weeks.

The pubescent female is generally attached with the adult male by copulatory anal suckers during both the active and quiescent phases of the pubescent female stage (up to 8 days). Copulation, however, does not occur during this long period of attachment, but takes place between the adult male and the ovigerous female at a precise moment during the final molt of the female. Eggs are not laid parthenogenetically. Bred ovigerous females, under the *in vitro* conditions, lay their eggs singly at the rate of about one a day for periods of 3 to 16 days. Females frequently return to common sites to oviposit. The period of embryonation after the egg is laid is usually 4 days. The oviposition period is followed by a period of senescence that lasts from 1 to 5 days.

Chorioptic mites from five hosts (cow, horse, goat, sheep, and llama), the first four representing four species of *Chorioptes* (*C. bovis*, *C. equi*, *C. caprae*, and *C. ovis*), were shown (1) to survive *in vitro* on each others' epidermic debris and complete the life cycle in the standard 3-week period; (2) to cross-breed and produce fertile progeny in the second generation; (3) to display similar survival periods on foreign epidermal material (three species of oxen, two equids, and three camelids); (4) to display almost identical results on epidermal material from some antelopes, which, in the family Bovidae, link the oxen on the one hand to the sheep and goats on the other; (5) to transmit from one host species to another; and (6) to display no morphological differences. Because of these data, the four species above are declared synonymous. The name with priority is *C. bovis*.

*Specimens from the present study have been deposited in the British Museum of Natural History where there is also the holotype (?) of *C. texanus*.

Chorioptic mites are potentially ubiquitous. The life cycle was completed *in vitro* on epidermal material from some, but not all, individuals of reindeer, wapiti, and white-tailed deer in Cervidae; impala, nyala, and eland among the antelopes; Indian water buffalo and zebu among the oxen; and the donkey and Grant's zebra in Equidae. Prolongation of the early developmental stages with premature death also occurred *in vitro* on epidermic debris from a wide variety of wild ungulates. Survival appeared to be related to the individual rather than the species. From these and other data, three of the four remaining species or subspecies of *Chorioptes* were also synonymized with *C. bovis*. This leaves in the genus *C. bovis* (Héring, 1845) Gervais and Van Beneden, 1859 and *C. texanus* Hirst, 1924.

The susceptibility of different breeds of domestic stock was tested. The life cycle was completed *in vitro* on epidermal material from 12 of 14 cattle breeds, 2 of 3 goat breeds, 3 of 15 horse breeds, and 5 of 13 sheep breeds. Domestic cattle appear to be the important host species, and probably have been most significant in the cosmopolitan distribution of the parasite.

Acknowledgments

It is a pleasure to acknowledge the advice and encouragement of Dr. Chas. A. Mitchell, former Chief, Animal Pathology Division, Canada Department of Agriculture, and of Prof. T. W. M. Cameron, Head, Department of Parasitology, McGill University. I am grateful also to Dr. J. W. Pullin, who assisted in various ways concordant with his position as Officer-in-Charge of the Montreal Area Branch Laboratory of the Animal Pathology Division, Canada Department of Agriculture. Mr. D. V. Conlon assisted in the field and with the conduction of the experiments, and Mr. J. Mackie assisted in the preparation of the final drawings. Mr. W. Goulet ably maintained the experimental animals. Material was obtained from various ungulates through the courtesy of numerous farmers, veterinary practitioners, governmental veterinarians, and officials of the London Zoological Society; New York Zoological Society; Société Zoologique de Granby; Jardin Zoologique de Québec; Smithsonian Institution, National Zoological Park, Washington; the Chicago Zoological Society; and the Institute of Northern Agricultural Research, Huntington Center, Vermont.

References

1. Baker, E. W. and Wharton, G. W. An introduction to acarology. The Macmillan Co., New York. 1952.
2. Brumpt, E. Précis de parasitologie. Vol. 2. Masson et Cie., Paris. 1936.
3. Buxton, P. W. The measurement and control of atmospheric humidity in relation to entomological problems. Bull. Entomol. Research, **22**, 431–447 (1932).
4. Cave, T. W. The foot-scab mite of sheep (*Symbiotes communis* var. *ovis*, Railliet). J. Comp. Pathol. Therap. **22**, 50–52 (1909).
5. Delafond, O. and Bourguignon, H. Recherches sur les animalcules de la gale de l'homme et des animaux et la transmission de la gale des animaux à l'homme. Bull. Acad. Méd. (Paris), **23**, 110–126 (1857-58).
6. Delafond, H. M. O. and Bourguignon, H. M. Traité pratique d'entomologie et de pathologie comparées de la psore ou gale de l'homme et des animaux domestiques. Mém. acad. sci. instit. France. Sci. Math. Phys. **16**, 277–922 (1862).

7. GERLACH, A. C. Krätze und Räude, entomologisch und klinisch bearbeitet. August Hirschwald, Berlin. 1857.
8. HELSON, G. A. H. Some arthropods affecting man and livestock in New Zealand. New Zealand Vet. J. **4**, 11–18 (1956).
9. HÉRING, E. Württemberg. Naturwiss. Jahresh. I *and* Repertorium der Thierheilkunde VI (1845).
10. HIRST, S. Mites injurious to domestic animals. (With an appendix on the acarine disease of hive bees.) Brit. Mus. (Nat. Hist.), Econ. Ser. No. 13. 1922.
11. HIRST, S. On a new mite of the genus *Chorioptes* parasitic on goats in the United States. Ann. Mag. Nat. Hist. **13**, 538 (1924).
12. KEMPER, H. E., ROBERTS, I. H., and PETERSON, H. O. Tests with benzene hexachloride on chorioptic scab mites. Sheep and Goat Raiser, **32**, 56–58 (1952).
13. MCKENNA, C. T. and PULSFORD, M. F. A note on the occurrence of *Chorioptes communis* var. *ovis* on sheep in South Australia. Australian Vet. J. **23**, 146–147 (1947).
14. MÉGNIN, J. P. Mémoire sur un nouvel acarien psorique du genre Symbiote. J. Anat. et Physiol. **8**, 337–358 (1872).
15. MÉGNIN, J. P. Monographie de la tribu des sarcoptides psoriques qui comprend tous les acariens de la gale de l'homme et des animaux. Deuxième Partie. Histoire naturelle des acariens qui constituent la tribu des sarcoptides psoriques. Rev. Mag. Zool. Pur. Appl. **40**, 82–173 (1877).
16. MOLLEREAU, M. A. Gale symbiotique de la chèvre. Bull. Soc. Centr. Méd. Vét. **43**, 156–157 (1889).
17. MURRAY, M. D. and THOMAS, P. L. Personal communication. 1956.
18. NEVEU-LEMAIRE, M. Traité d'entomologie médicale et vétérinaire. *Edited by* Vigot Frères. Paris. 1938.
19. OUDEMANS, A. C. *Chorioptes caprae* (Del. & Bourg. 1858). Tijdschr. Entomol. s'Gravenhage, **69**, 1–18 (1926).
20. PALIMPSESTOV, M. A. [Peculiarities in the development of psoroptid mange mites.] (In Russian.) Vet. **24**, 6–9 (1947).
21. PILGRIM, G. E. The fossil Bovidae of India. Paläontol. Indica, N.S. **26**, 1–356 (1939).
22. RADFORD, C. D. The mites (Acarina) parasitic on mammals, birds and reptiles. Parasitology, **40**, 366–394 (1950).
23. RAILLIET, A. Traité de zoologie médicale et agricole. Part I. Asselin et Houzeau, Paris. 1893.
24. RAILLIET, A. and MOUQUET, A. E. Gale chorioptique du mouflon à manchettes. Bull. Soc. Centr. Méd. Vét. **72**, 212–215 (1919).
25. ROSE, A. L. Leg mange or "itchy heel" of horses. Description of the disease and methods of treating it. Agr. Gaz. N.S. Wales, **51**, 491–496 (1940).
26. SWEATMAN, G. K. Seasonal variations in the sites of infestation of *Chorioptes bovis*, a parasitic mite of cattle, with observations on the associated dermatitis. Can. J. Comp. Med. Vet. Sci., **20**, 321–336 (1956).
27. VITZTHUM, H. G. Acarina *in*, Klassen und Ordnungen des Tierreichs, 5. Band: Arthropoda, IV Abteilung: Arachnoidea, 5. Buch. *by* H. G. Bronn. Becker & Erler Kom.-Ges., Leipzig. 1943.
28. ZÜRN, F. A., Kurze Mittheilung über den sog. Salzfluss der Schafe. Wochschr. Tierh. u. Viehzucht, **18**, 121–122 (1874).
29. ZÜRN, F. A. Räudemilben im Ohr der Hunde und bei Kaninchen. Wochenschr. Tierh. u. Viehzucht, **18**, 277–283 (1874).

EFFECTS OF DIETARY PHOSPHOLIPID AND CHOLESTEROL ON THE TEMPERATURE RESISTANCE OF GOLDFISH[1,2]

D. G. Irvine, Katherine Newman, and W. S. Hoar

Abstract

The temperature resistance of goldfish is increased by additions of cholesterol or phospholipid to basic diets of pablum or pablum and pilchard oil. The relative effectiveness of these treatments varies with the season and with the duration of the feeding experiment. In low-temperature resistance tests, the cholesterol effect was most marked during the earlier period of chilling and the suggestion is that cholesterol increases the capacity of fish to resist cold narcosis—not death. In heat resistance tests, the phospholipid-fed fish showed a tendency toward heat narcosis which was not evident with cholesterol-fed animals. Increased temperature resistance was closely correlated with increased tissue cholesterol but only weakly correlated with the less modified tissue phospholipid. Although these diets also produced definite changes in total lipid content, moisture content, unsaturation of total lipids, and the cholesterol/phospholipid ratio, no evident correlation between these factors and temperature resistance of the goldfish was found. Data are discussed in relation to several theories of temperature death.

Introduction

It has been known for many years that acclimatization to cold frequently increases the unsaturation of the tissue lipids whereas acclimatization to heat causes the lipids to become less unsaturated and hence more solid (4, 13). Diet-evoked changes in unsaturation of total body lipids of goldfish, however, brought about neither consistently increased cold resistance with increased unsaturation nor regularly enhanced heat resistance with increased saturation (8, 10). Although dietary lipids had a marked effect on thermal resistance, there was no close correlation between total lipid unsaturation and resistance to sudden changes in temperature.

Several workers have studied changes in tissue cholesterol and phospholipid of animals acclimatized to different temperatures (11, 12, 15, 16). Acclimatization of goldfish to low temperatures in this laboratory decreased the tissue phospholipids but had no significant effect on tissue cholesterol (9). In other words, there was a marked rise in the ratio of tissue cholesterol to phospholipid (C/P ratio) with acclimatization to low temperatures. When, however, in preliminary experiments, an attempt was made to correlate diet-induced changes in thermal resistance with the C/P ratio the most cold-resistant fish had the lowest values. Thus, among the acclimatization groups, fish with the highest C/P ratio and the lowest tissue phospholipids were most resistant to cold but among the diet groups the most resistant fish had the lowest C/P ratio and the highest phospholipid

[1]Manuscript received May 13, 1957.
Contribution from the Department of Zoology, The University of British Columbia, Vancouver, British Columbia, with financial assistance from the National Research Council of Canada.

[2]Data on low temperature resistance and on the tissue chemistry are taken from a thesis presented by D. G. Irvine in partial fulfillment of the requirements for the degree of Master of Arts in Zoology.

values. Clearly the matter required further examination. The present paper is an attempt to evaluate the effects of dietary phospholipid and cholesterol on both the heat and cold resistance of goldfish.

Materials and Methods

Goldfish (*Carassius auratus*) were obtained in lots of from 200 to 500 per shipment from the Goldfish Supply Co., Stouffville, Ontario. They were maintained in the laboratory at approximately 20° C. and fed on standard diets supplemented with different lipids. Details for the care of the fish and for the preparation of diets have been given elsewhere (6, 10). Pertinent data as to number of animals, size, sex, and time of study are given in Table I.

TABLE I

FISH USED IN TEMPERATURE RESISTANCE EXPERIMENTS

Shipment no.	Diets	Days of feeding	Test	Date of test	No. of fish	Mean weight, g.	Females per 1 male
I	A,B,C,D	40	Cold	Nov. 24/52	40		
		42	Cold	Nov. 26/52	40		
		44	Heat	Nov. 28/52	46		
II	P,E,F	45	Cold	June 19/53	51	8.8	
		25	Cold	June 22/53	51	9.2	
		65	Cold	July 9/53	40	9.9	0.80
		46	Cold	July 12/53	51	9.2	0.60
III	A,B,C,D	24	Cold	Aug. 14/53	69	9.9	0.81
		38	Cold	Aug. 29/53	72	10.9	0.91
IV	P,E,F	7	Cold	Nov. 4/53	51	12.3	1.23
		14	Cold	Nov. 11/53	50	12.1	1.27
		21	Cold	Nov. 18/53	51	14.1	0.47
V	P,E,F,	25	Heat	June 10/55	90	12.2	1.07
		41	Heat	June 26/55	68	11.7	1.06
VI	P,E,F	7	Heat	Aug. 8/55	60	9.6	0.84
		14	Heat	Aug. 15/55	60	9.4	1.04
		28	Heat	Aug. 29/55	57	10.1	0.86
				Total	947		

Composition of the Diets

The data recorded here are based on two types of high lipid diet. In the first series, varying amounts of lecithin and/or cholesterol were added to a basic diet of pablum and pilchard oil. In this series an attempt was made to prepare diets comparable to the hydrogenated pilchard oil series (8) but differing in cholesterol or phospholipid. In the second series the diets were simplified by the omission of the pilchard oil. Details for the composition of the diets are given in Table II. Only the cholesterol is a chemically pure material. The lecithin is an animal product while the "granulestin" is prepared from soybeans. The composition of "granulestin", as given by the supplier, is shown in Table II. The diets are complex and, with the

phospholipids in particular, it is impossible to say which of the constituents may be involved in the temperature resistance changes described. The arguments, therefore, are based on correlations of temperature resistance with the changes induced in tissue cholesterol and phospholipid of the goldfish.

TABLE II

COMPOSITION OF DIETS

Diet	Constituents (g.)				
	Pablum*	Pilchard oil†	Cholesterol‡	Lecithin§	Granulestin‖
A	70	25	–	5	–
B	70	18.75	6.25	5	–
C	70	20	–	10	–
D	70	15	5	10	–
E	50	–	50	–	–
F	50	–	–	–	50
P	100	–	–	–	–

*Mixed cereals, Mead Johnson & Co.
†Van Camp Sea Foods, California.
‡Fisher Laboratory Chemical.
§Animal lecithin (practical), Eastman Kodak Company.
‖80% phospholipids (mixture of lecithin, cephalin, and inositol phosphatide), American Lecithin Company.

Testing Thermal Resistance

No attempt has been made to provide a complete picture of the temperature tolerance (3) of the different groups of goldfish. Rather, the attempt has been to evaluate the effects of the different diets by subjecting the groups under comparison—at the same time and in the same lethal temperature bath—to a standard sudden change in temperature which would kill most of the fish in a convenient period of time (5 to 24 hours). Low temperature death was produced by placing the fish in a bath at approximately 2° C. and cooling this gradually to about 1° C. over a period of 5 to 10 hours. The effects of high temperature were compared in a bath maintained at 35.5 ± 0.5° C. No fish was removed from the low temperature bath during the first hour. Thereafter, fish were permanently removed when they no longer responded to standard mechanical stimulation of the caudal peduncle—first while in the cold water and then in air upon a cold wet glass plate. Fish were removed from the high temperature bath as soon as they failed, while in the tank, to respond to tapping with a glass rod. In either case the animals permanently removed ("removals") were placed in a 20° C. bath to determine whether they were narcotized or truly dead. After about one hour the fish were autopsied and the size and sex recorded.

Low temperature mortality reported previously (8) was the sum of two factors—deep cold narcosis and true death due to chilling. In the present study an attempt has been made to distinguish these two factors. This is necessary since not only diet but also sex has been found to influence both

factors. Although criteria for removal from the cold tank were precisely defined, it was sometimes found difficult to tell just when a fish should be removed. There is thus an obvious potential source of bias. For this reason, the different groups of fish were placed in the different compartments of the cold tank by one of the investigators and removed by another. Thus, the person making the crucial determination of whether or not a fish should be removed was unaware of its dietary history until the data were assembled at the end of the test.

Chemical Analyses

Moisture and lipid determinations of whole macerated goldfish tissues were carried out on samples of the control and fat-fed fish which had not been subjected to the lethal temperature tests. In an earlier study (9) fish were analyzed after they were killed by temperature exposure and this may have led to inconsistencies in the data.

Moisture was determined with the Cenco moisture balance at reduced voltage. Average triplicate determinations were checked against the conventional oven-drying method and found to agree within a maximum of 0.28%. Determinations can be completed in 25 minutes and it is doubtful whether the added precision afforded by the oven-drying is justified in an investigation such as this.

Total lipids were determined by extracting minced whole goldfish (one part goldfish to two parts anhydrous sodium sulphate) with Bloor's mixture (three parts ethyl alcohol to one part anhydrous peroxide-free diethyl ether). The use of Bloor's mixture in place of ether ensures more complete lipid extraction from tissues. However, material extracted in this way is not all ether-soluble (e.g. glycolipids) and, to obtain results qualitatively comparable with those reported previously (9), the weighed Bloor's solubles were exhaustively extracted with warm ether, the resulting loss in weight being reported as "total lipid". This indirect procedure for the estimation of "ether solubles" was adopted initially in an attempt to study changes in constituents removed by Bloor's mixture but not by ether. However, no relationships with temperature resistance were evident and all data are, in fact, based on ether extracts.

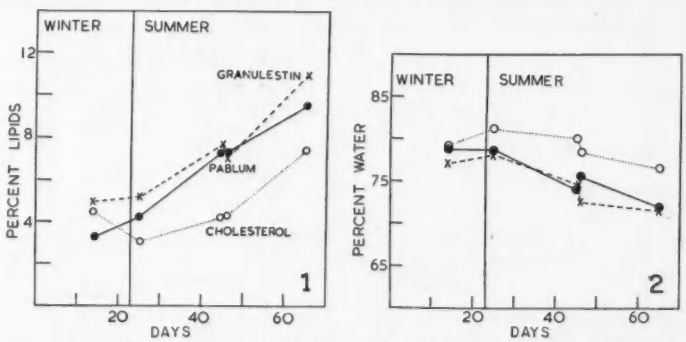
Cholesterol, iodine number, and phospholipid were determined on direct ether extracts of minced tissues. Tissues were minced with anhydrous sodium sulphate and peroxide-free, redistilled ether in a Waring Blendor. The routine Liebermann-Burchard method was used for the estimation of cholesterol. Iodine numbers were determined by the Wij method using 100 ± 10 mg. lipid. The phospholipid method was considerably modified from the original Bloor technique (1). Phospholipids were precipitated in the absence of magnesium chloride using 9 ml. of chilled acetone to 3 ml. of the ether extract of lipids. The mixture was chilled for 30 minutes in ether-washed–preweighed 15 ml. conical centrifuge tubes, centrifuged, the supernatant discarded, the precipitate washed with chilled acetone, centrifuged once more, and the precipitate dried *in vacuo* and weighed. The precipitates

contained less than 0.015 mg. cholesterol per mg. phospholipid and this is within the limits of accuracy of this method. The supernatant contained less than 0.06 mg. phospholipid and could be used for the cholesterol determinations.

Results

Effects of Dietary Lipids on Tissue Lipids

Tissue lipids were determined in goldfish fed on pablum (control) and on diets of pablum with 50% cholesterol or 50% granulestin. All diets produced a progressive increase in total lipids of the tissues with a concomitant decrease in the moisture content (Figs. 1 and 2). This inverse relationship between percentage of lipids and percentage of water in the tissues has been apparent not only in these dietary studies but in other biochemical work carried out on the goldfish in this laboratory. Data for control goldfish (pablum-fed) used in a number of different investigations are summarized in Fig. 3.



FIGS. 1 and 2. Effects of diets of pablum (solid line), pablum supplemented with 50% cholesterol (dotted line), or 50% granulestin (broken line) on the lipid (Fig. 1) and water (Fig. 2) content of (wet) goldfish tissues.

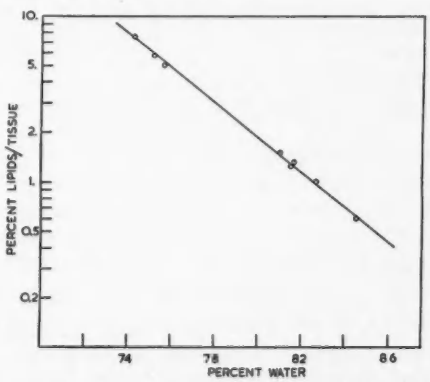


FIG. 3. The inverse relationship between tissue water and ether extractables in goldfish on a diet of pablum; different seasons and different acclimatization temperatures; semi-logarithmic plot.

Tissue lipid changes produced by prolonged feeding of the pablum control diet are shown in Figs. 4, 5, and 6. This diet is 70% carbohydrate (3% lipid and 15% protein). After 4 weeks it induced a progressive saturation of the body fats, probably due to their synthesis from the carbohydrate (Fig. 4). Tissue phospholipids rose slowly during the 65 days of study while tissue cholesterol showed little change although the cholesterol content of the total lipid declined progressively (Fig. 5). The *C/P* ratio remained almost constant (Fig. 6).

The diet containing 50% cholesterol produced marked changes in tissue lipids. Total lipid was relatively lower and water content relatively higher than in fish feeding on the control diet or the high phospholipid ration (Figs. 1 and 2). The tissue cholesterol and phospholipid values show an orderly trend during the first 7 weeks followed by a return toward the original level at the time of final sampling (Fig. 7). This 65-day value is believed to represent a real change due in some way to prolonged feeding. Comparable

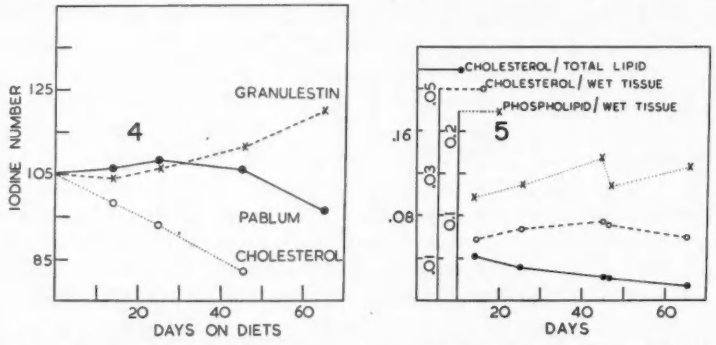


FIG. 4. Changes in total lipid unsaturation produced by diets of pablum (solid line), pablum supplemented with 50% cholesterol (dotted line), or 50% granulestin (broken line).

FIG. 5. Tissue lipid changes produced by the control diet of pablum expressed as percentage of total lipid or wet tissue.

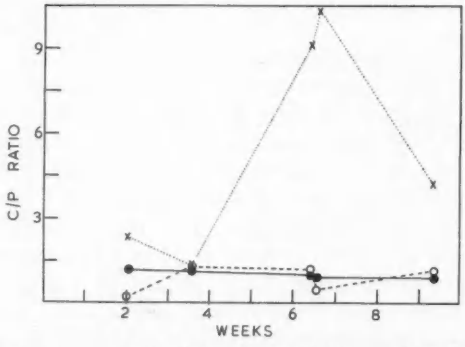
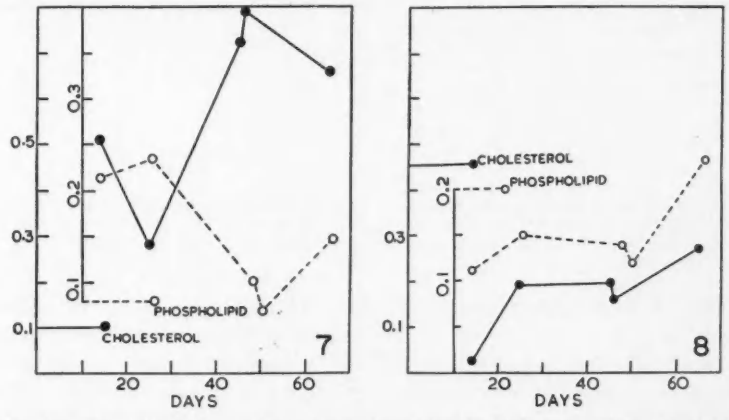


FIG. 6. Progressive changes in the *C/P* ratio of fish fed on pablum (solid line), pablum supplemented with 50% cholesterol (dotted line), or 50% granulestin (broken line).

trends in temperature resistance will be described below. In detail, the tissue cholesterol (Fig. 7) and the *C/P* ratio (Fig. 6) increased markedly reaching a peak at about 50 days and declining thereafter. The phospholipid (Fig. 7) declined from a peak during the initial period but was rising again at 65 days. High cholesterol diet produced a marked and steady decline in the unsaturation of the tissue lipids (Fig. 4).

Tissue changes produced by the diet containing 50% granulestin (soya phosphatides) were less marked than those in fish fed high cholesterol rations. The total lipid and moisture contents of the tissues were essentially the same as those of the controls (Figs. 1 and 2). Other tissue lipid changes are shown in Fig. 8. The initial cholesterol value is much lower than that of the control. Otherwise both cholesterol and phospholipid values did not differ greatly from those of the controls and showed a slow but somewhat irregular rise during the entire period. The ratios of cholesterol to phospholipid (Fig. 6) or to total lipid were similar to those of the controls. However, the degree of unsaturation rose steadily and deviated markedly from the control at the conclusion of the experiment (Fig. 4).



FIGS. 7 and 8. Tissue changes in cholesterol and phospholipid produced by a diet of 50% cholesterol (Fig. 7) and 50% granulestin (Fig. 8) expressed as per cent in wet tissue.

In summary, these diets all produced progressive fattening of the goldfish coupled with proportional water loss. Pablum alone produced small specific effects. Cholesterol caused an absolute decrease in the iodine number, a relative decrease in total lipid, and a relative increase in water of the tissues. The iodine number rose in the phospholipid-fed fish but there was no particular change in the relative amounts of lipid and water in the tissues. The effects of these diets on the tissue cholesterol and phospholipid can be seen more readily when the data are plotted in another way. In Fig. 9 the control (pablum) values are taken as unity. The progressive changes of cholesterol in the lipid (Fig. 9A) and of phospholipid in the wet tissue (Fig. 9B) are plotted as departures from this against days of feeding. The cholesterol ration induced

a marked rise in cholesterol (Fig. 9) and a high initial phospholipid which subsequently declined (Fig. 9B). The tests at 9 weeks show a reversal in trends. The high phospholipid ration produced an initial fall in the cholesterol with a prompt return to normal and little variation thereafter (Fig. 9A). This diet did not change the percentage of tissue phospholipids. The variation about the control value was as often in one direction as the other (Fig. 9B).

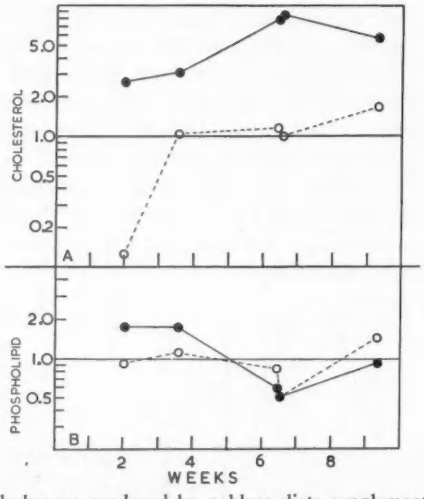


FIG. 9. Chemical changes produced by pablum diets supplemented with cholesterol (solid line) or granulestin (broken line), expressed as ratios of the control values for pablum-fed fish. A, cholesterol in total lipids; B, phospholipids in (wet) goldfish tissues; semilogarithmic plots.

Table III summarizes tissue lipid data for diets containing 30% herring oil, pilchard oil, or lard. The thermal history of these fish has been reported elsewhere (8) and the analyses will be considered below in the general discussion. Table III was compiled by Merva K. Cottle and the analytical techniques (9) differed somewhat from those used in the work described above.

TABLE III

ANALYSES OF GOLDFISH TISSUES AND EXTRACTED OILS (DATA OF MERVA K. COTTLE)

Diet	Water, %	Oil, %	Iodine value	Phospholipid, % in oil	Cholesterol, % in oil	Cholesterol/ phospholipid ratio
Pilchard oil	74.6	3.86	137	0.77	6.5	8.54
Herring oil	75.0	4.23	115	3.96	4.9	1.23
Lard	75.0	4.97	84	0.75	3.4	4.52
Hydrogenated pilchard oil	79.6	2.05	107	5.7	6.8	1.19
	77.3	2.42	112	3.6	5.4	1.5
	76.8	–	95	2.6	3.9	1.5
	76.6	2.5	90	2.3	5.4	2.34

Resistance to Temperature

Low Temperature Tests

Lipid supplements of pilchard oil with added cholesterol or lecithin (Diets A, B, C, D in Table II) were fed both during the summer and the winter. The data in terms of both 25% and 50% removal and true death times are summarized in Table IV. It should be emphasized that the data given for different dates of testing in this table are not comparable since the initial temperature of the bath varied (see Methods). However, on any one date the fish from the different diet groups were exposed together and are strictly comparable. Only those differences found in fish subjected to an identical temperature history are discussed here.

TABLE IV

EFFECTS OF SUPPLEMENTS OF PILCHARD OIL AND/OR CHOLESTEROL AND/OR LECITHIN ON COLD RESISTANCE. DEVIATIONS ARE DIFFERENCES BETWEEN RESISTANCE TIMES FOR FISH FED DIET A (CONTROL) AND DIETS B,C,D. SURVIVAL, S.

Diet	Days of feeding	25% time, hours				50% time, hours			
		Removed	Deviation	Dead	Deviation	Removed	Deviation	Dead	Deviation
Aug. 14 A	24	10		14		15		21	
B		10	0	12	− 2	14	− 1	19	− 2
C		7	− 3	14	0	15	0	17	− 4
D		17	+ 7	16	+ 2	21	+ 6	23	+ 2
Aug. 29 A	38	9		13		15		17	
B		10	+ 1	13	0	15	0	16	− 1
C		15	+ 6	17	+ 4	19	+ 4	20	+ 3
D		26	+ 17	29	+16	31	+16	35	+18
Nov. 24 A	40	<2		2		9		15	
B		4	+ 3	13	+11	11	+ 2	26	+11
C		4	+ 3	23	+21	4	− 5	38	+23
D		<2	0	4	+ 2	23	+14	29	+14
Nov. 26 A	42	1.5		3		3		24	
B		2	+ 0.5	24	+21	3	0	70%S	+++
C		3	+ 1.5	24	+21	16	+13	24	0
D		2.5	+ 1.0	16	+13	4	+ 1	34	+10

Despite the complexity of these diets, several features are evident in the cold resistance of the fish. Diet A is the control diet of earlier work on pilchard oil (8, 10) and in general, fish feeding on it showed the poorest cold resistance. Diet B, containing the most cholesterol, produced little change in the cold resistance of summer fish but markedly increased that of the winter fish. Diet C, containing the maximum amount of lecithin but no additional cholesterol, also increased the cold resistance of winter fish (maximum winter effect of the diets of this series) and, to a lesser degree, that of the summer fish. Diet D, containing supplements of both lecithin and cholesterol was consistently beneficial both summer and winter. Thus, increased chill resistance is apparently associated with cholesterol supplements in the winter and with lecithin supplements both summer and winter.

The cholesterol effect may, however, be looked at in another way. Diet B is pablum supplemented with cholesterol, while Diet D is pablum and lecithin supplemented with cholesterol. If we examine the effect of cholesterol supplement alone (B with A) it appears that cholesterol is active only in the winter, while, if we compare the effect of adding cholesterol to a lecithin-supplemented diet (D with C), it appears that cholesterol is more active during the summer (see Table IV). A seasonal variation in thermal resistance has been described (6, 7) and the indications are that the level of phospholipid may vary the seasonal response to cholesterol. Fig. 10 summarizes the effects of supplements of lecithin and cholesterol on pilchard oil diets fed to summer fish.

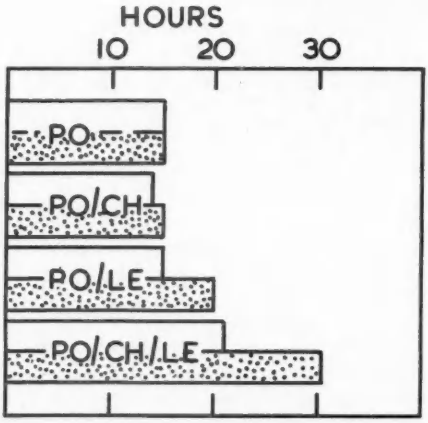


FIG. 10. Effects of cholesterol or lecithin supplements on the cold resistance of goldfish during the summer—expressed as removal times for 50% of the samples; *PO*, pilchard oil diet with supplements of *CH*, cholesterol and/or *LE*, lecithin; open bars, feeding for 24 days; stippled bars, feeding for 38 days.

Table III supports the above evidence that pilchard oil diets are by themselves less effective in cold resistance than diets of pilchard oil supplemented by phospholipid and cholesterol. It has already been shown (8) that the diets containing hydrogenated pilchard oil become progressively less effective in chill resistance as the iodine value of the oil declines (less unsaturation). Table III shows that the phospholipids fall markedly with the iodine value. Changes in the cholesterol content are less but the same trend is present. Of the natural fats listed in Table III, herring oil is a much more effective dietary supplement for the promotion of chill resistance than either pilchard oil or lard. The phospholipid content of the herring oil is about five times as great as that of the other two fats and the cholesterol content almost 1.5 times greater than that of lard. These data are fully in accord with those presented in Table IV.

Goldfish were maintained on diets of pablum and 50% cholesterol or 50% granulestin (Diets P, E, F, Table II) for prolonged periods and the chill resistance tested at intervals. The effectiveness of cholesterol in counteracting

chill death or chill narcosis is again evident (Fig. 11). Data with respect to "removals" follow an orderly trend. Resistance is increased between the second and seventh week, whether the data are considered in terms of 25% or 50% removal times. Prolonged feeding of these diets produces detrimental effects.

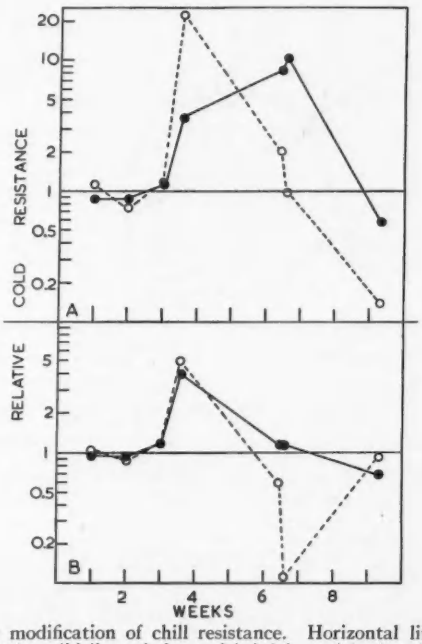


FIG. 11. Dietary modification of chill resistance. Horizontal lines at 1 represent control pablum-fed fish; solid line, cholesterol-fed fish, and broken line, granulestin-fed fish; A, time required for 25% removal referred back to the unit of time it took for 25% removal of the respective controls; B, 50% removal times calculated in the same way; semilogarithmic plot.

The data for the truly dead cholesterol-fed fish are less consistent and the effect can only be interpreted as a negative or a variable one. The suggestion is that cholesterol increases the capacity of fish to resist cold narcosis—not death. This effect can be brought out more clearly in detailed comparisons of the action of dietary cholesterol during the early period of chilling. Differences not evident in a two-point comparison, such as Table IV, are apparent in Fig. 12. In this figure the pablum–pilchard oil diets, as well as those presently under consideration, are compared. The inner band of Fig. 12 represents the benefit from cholesterol feeding (in percentage spared) afforded at 150 minutes' exposure to cold. This effect is absent at 15 hours (outer band). This "initial protection" due to cholesterol is rather variable in its duration but rarely fails to appear.

The effects of prolonged granulestin feeding are also shown in Fig. 11. With this supplement, likewise, the cold resistance was greater for several weeks but at the end of the experiment the fish were considerably less resistant

than the controls. The effect is most evident in terms of 25% removal times (Fig. 11A), again indicating that the lipid supplement is especially beneficial in the initial stages of chilling.

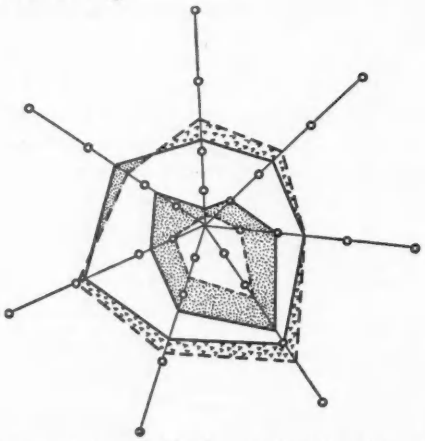


FIG. 12. Dietary cholesterol and "initial protection" in chill. Circles in order from center show 20, 40, 60, and 80% mortality. The seven axes show the effects of high cholesterol diets fed to seven different groups of fish for periods ranging from 25 to 45 days. Solid lines joining axes, controls; broken lines, cholesterol-fed fish; inner stippled band, protective effect at 2½ hours' exposure; outer band ("v" marks), variable but usually slight or detrimental effect at 15 hours' exposure.

High Temperature Tests

Goldfish fed supplements of pilchard oil with added cholesterol or lecithin (Diets A, B, C, and D, Table II) were subjected to only one high-temperature resistance test. These were winter fish. The marked protective action afforded by cholesterol is evident both in terms of 25% or 50% true mortality times (Table V).

Goldfish that were fed pablum supplemented with 50% cholesterol or 50% granulestin were tested for high temperature resistance at intervals over a period of 42 days. Only summer fish were studied. These would be expected to have a relatively high initial resistance to heat and changes produced would be particularly significant. The results are summarized in Figs. 13 and 14.

TABLE V

EFFECT ON HEAT RESISTANCE OF ADDING CHOLESTEROL OR LECITHIN SUPPLEMENTS TO A PABLUM DIET CONTAINING 25% PILCHARD OIL: NOVEMBER 28, 1952; 44 DAYS ON DIETARY REGIME; TEST TEMPERATURE 35.0–35.5° C.

Diet	Time (min.) to cause true death in:	
	25% sample	50% sample
A Control	60	140
B + cholesterol	240	405
C + lecithin	45	160

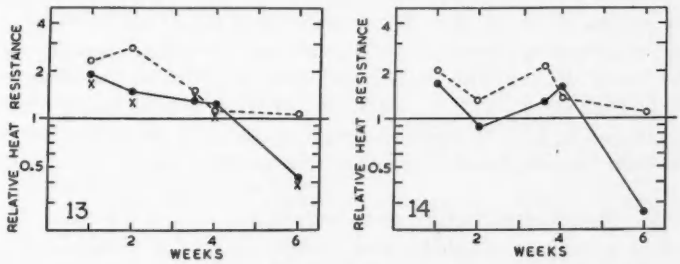


FIG. 13. Heat resistance of cholesterol-fed fish, expressed as times required to remove (trend lines) or to kill (× marks) 50% of the sample referred back to the unit of time for 50% of the respective controls. Horizontal line at 1, control pablum-fed fish; solid trend line, 50% removal at test temperature of 35.0–35.5° C., broken line, 50% removal at test temperature of 35.8–36.4° C., ×, 50% truly dead at test temperature of 35.0–35.5° C.; semilogarithmic scale.

Fig. 14. Heat resistance of granulestin-fed fish, expressed as in Fig. 13. Solid trend line, 50% removed at test temperature of 35.0–35.5° C.; broken line, 50% truly dead at test temperature of 35.0–35.5° C.; semilogarithmic scale.

The changes induced by cholesterol are marked and follow the same general trend noted in chill resistance. That is, with increasing duration of dietary pretreatment, the earlier increased protection is followed later by a decline in resistance to heat. This is true whether the results are expressed in terms of removals or truly dead fish and evident at test temperatures of both 35° and 36° C. (Fig. 13). The narcosis effect noted in the low temperature resistance tests is not evident when the cholesterol-fed and control fish are killed with heat.

Granulestin also increased the resistance of the goldfish to heat (Fig. 14). In this case the picture in terms of removals is irregular although the resistance is in general high during the first 4 weeks but markedly lower in the final 6-week test. In terms of truly dead fish, the curves follow a similar trend but resistance is always higher in the experimental than in the control animals. Differences between removal times and times for true death are much greater with the granulestin than with the cholesterol-supplemented diets (Figs. 13, 14). The granulestin-fed fish were thus more likely to enter heat narcosis than were the cholesterol-fed animals.

In summary, diets containing 50% cholesterol or 50% granulestin increased the heat resistance of goldfish during the first 2 or 3 weeks of feeding but produced detrimental effects with prolonged administration (6 weeks). Differences between removal times and times for true death were evident in fish fed the phospholipid diet but not in those fed the cholesterol diet.

Discussion

The data show clearly that dietary supplements of cholesterol or phospholipid increase both the high and low temperature resistance of goldfish. The central question arising from this demonstration concerns the mechanism by which these effects are brought about, and the significance of cholesterol and phospholipid in temperature narcosis and death. Another problem apparent

in these results concerns the characteristic time-resistance curves produced by some of the dietary regimes. In most cases, the increased resistance is followed by a decline with continued feedings. Before the data can be discussed in relation to temperature death, however, it is necessary to evaluate several possible sources of bias since season, size, and sex, as well as dietary history, have been shown to affect the temperature resistance of goldfish (6).

Non-dietary Factors and Temperature Resistance

Seasonal variations in temperature resistance of fish, maintained at the same temperature and fed a standard diet, have been noted over a period of 5 years. The findings are discussed elsewhere (6). Seasonal changes are probably photoperiodically controlled (7) and, in nature, confer a certain temperature independence on fish which may be exposed to sudden environmental changes in the spring and fall. In the present paper, this factor is controlled by confining the comparisons to groups treated at the same time and tested together in the same lethal bath.

Size and sex affect both cold and heat resistance of goldfish. In an earlier paper (6), it was shown that the average weight of goldfish removed in the early part of a cold test was always less than that of fish removed later and that for each centimeter increase in length an added 2 hours' survival time might be expected. Further, male fish are more resistant to cold than female fish. In the present series of experiments, it was evident that high temperature resistance was also modified by size and sex of the fish. In the heat tests (Table VI), the larger fish regularly die before the smaller fish; the females die somewhat earlier than the males (Table VII). The size effect seems definite enough but the action of sex is only an indication in these data. In the high-temperature resistance tests, fish die so rapidly that factors which confer relatively great advantages in nature, where changes are more gradual, might be largely masked. In the cold test the selective action of such factors as size and sex is spread over a longer period and thus the effects are more evident in the results.

TABLE VI

MEAN WEIGHT (G.) OF GOLDFISH REMOVED DURING DIFFERENT PERIODS OF HIGH TEMPERATURE EXPOSURE

	Removals					
	0–60 min.		60–120 min.		120 min.	
Diet group	No.	Wt.	No.	Wt.	No.	Wt.
Pablum control	55	11.7	22	11.4	35	11.3
Cholesterol 50%	47	11.5	23	10.4	42	10.1
Granulestin 50%	63	10.8	18	9.2	29	9.4
Total	165	11.3	63	10.4	106	10.3

TABLE VII

SEX RATIOS OF GOLDFISH USED FOR HIGH TEMPERATURE RESISTANCE TESTS—JUNE TO AUGUST, 1955

	Fish removed during					
	0–60 min.		60–120 min.		120 min.–end	
	Male	Female	Male	Female	Male	Female
Pablum diet	27	28	13	9	17	14
Cholesterol supplement	23	24	9	14	21	19
Granulestin supplement	29	32	12	6	13	15
Total	79	84	34	29	51	48
Total 164 males: 161 females						
Females/1 male	1.07		0.85		0.94	

These non-dietary differences are doubtless responsible for some of the obvious irregularities in results. It was not practical to use a sufficiently large number of fish in the different tests to calculate age- and sex-specific mortality rates. The sex of a goldfish can usually be determined only by autopsy. Reasonable care was exercised to assign fish of the same general size range to the different tanks and to the compartments of the lethal bath. The bias introduced by the sex factor cannot invalidate the over-all conclusions since chill test removal times for winter fish are not subject to this effect (see Hoar (6, Fig. 5)) and since the sex differences are so small in the heat resistance tests (Table VI). Difference in resistance due to size in the different samples is usually much less than the differences in resistance times on which these conclusions are based. It is, then, recognized that variability in the sizes and sexes of the different individuals in the samples has introduced irregularities in the results but have not modified the general pattern and the conclusions.

Cholesterol and Phospholipid in Relation to Temperature Resistance

Different theories have been proposed for the primary disorganization in physiological processes which culminates in temperature death (4, 13). The present investigation does not point definitely to the correct theory or theories. It does, however, provide further evidence against an explanation based on the melting point of the body fat but suggests that certain lipid factors will find a prominent place in the explanation which eventually proves to be correct.

The tissue cholesterol/phospholipid ratio, which has been related by several workers to cell permeability and temperature resistance, shows very poor correlation with the differences in temperature resistance observed in this study. Hoar and Cottle (9) found that the C/P ratio declined and the cholesterol to fatty acid ratio rose with increasing heat resistance and with increasing water content of the tissues during temperature acclimatization experiments. These changes were opposite to those reported by Wilbur and

Del Pomo (16) for differences between Arctic and non-Arctic fish and to those of Mayer and Schaeffer (11, 12) for water content of tissues of several different animals. It has already been pointed out, however, that the analyses of the acclimatized goldfish were carried out on fish killed in the lethal tests and may not have represented the actual relationships in the living animals. In the present study separate samples have been used for tissue chemistry and for temperature resistance tests, yet, no consistent correlation has been found between the *C/P* ratio and either water content of the tissues or thermal resistance of the fish.

In our data, prolonged feeding of cholesterol is associated with increasing cholesterol content of the tissue lipids for a period of several weeks after which it declines (Figs. 7 and 9). Resistance to both heat and cold follows a similar trend (Figs. 11 and 13). During the entire period the water content falls progressively (Fig. 2). Likewise, water content did not seem to be correlated with temperature resistance when the diets were supplemented with phospholipid (Figs. 2, 11, and 14). The actual *C/P* ratio declined slightly in the pablum-fed control fish, rose in those receiving 50% cholesterol, and followed the control values in those given 50% granulestin (Fig. 6). Moreover, fish fed on pilchard oil, herring oil, or lard show no definite correlation between temperature resistance and the *C/P* ratio (Table III). In these groups, herring-oil-fed fish are most resistant to cold (low *C/P* ratio), and the lard-fed fish most resistant to heat (intermediate *C/P* ratio), while in the hydrogenated pilchard oil series the lowest *C/P* ratio is associated with the highest resistance to both heat and cold (8, 10). Thus, the actual content of cholesterol or phospholipid in the tissues is more definitely correlated with temperature resistance than is the ratio of these lipid constituents.

The melting of the lipids, with release of calcium and/or lipid from the all-important cell membrane, has been carefully considered in many discussions of temperature death (4, 13). The present investigation, as well as previous ones, shows that the melting point (as measured by the unsaturation) of the total body lipids is not correlated with resistance to either low temperature or high temperature death of these experimental fish. Temperature resistance associated with cholesterol feeding was marked by the development of progressively more solid fats (saturation) whereas the reverse (desaturation) was observed with temperature resistance conferred by the granulestin diet. Present studies, however, do not necessarily provide evidence concerning the situation with respect to the lipids in the cell membranes or the important lipids in the nervous tissue. Specific changes in the lipids of the cell membranes or nervous tissue would be masked by the relatively large amounts of depot fat and transport phospholipid.

Body insulation as such has been considered a factor modifying cold resistance of some animals. Despite the poikilothermy of the goldfish, it is conceivable that small differences in the rate of change of its body temperature might be critical. However, it has been previously mentioned that diet modifies cold resistance much more than either size or sex. Moreover, in terms of total tissue lipids, the pablum-fed control fish were insulated just as

well as, or better than, the cholesterol- and granulestin-fed fish (Fig. 1). Fig. 1 also shows that tissue lipids were still rising at 65 days of feeding while temperature resistance was showing a marked decline.

These dietary experiments add no information bearing directly on the different viscosity–coagulation theories, anoxia theories, or enzyme theories of temperature death. Progressive changes in temperature resistance with continued feeding of these diets is, however, marked and may be suggestive of the mechanism involved in temperature resistance.

In both heat and cold resistance tests the cholesterol-fed fish showed a pattern of high resistance followed by declining resistance after 6 weeks or more (Figs. 11, 13). The same trend is also evident in several of the resistance curves for fish fed phospholipid supplements (Figs. 11 and 14). Moreover, it may be significant that these goldfish show remarkably similar, although very complex, time resistance curves whether subjected to heat or exposed to cold (Figs. 11, 13, 14). To facilitate study of these time-resistance curves, three different working hypotheses are being considered: (1) that they are manifestations of Selye's (14) general adaptation syndrome, (2) that they exemplify Hickman's (5) physiological antioxidant activity curve in conditions of limiting concentration of some essential metabolite or oxygen, and (3) that the curves are due to some other complex change in metabolic pattern—possibly mediated through the endocrine system.

On the basis of mammalian physiology, it could be argued that a "stress" condition was induced in goldfish fed for prolonged periods on a diet of 50% cholesterol or phospholipid. However, the interrenal glands (comparable to the mammalian adrenal cortex) of these fish have been examined histologically and show no consistent evidence of hypertrophy or hyperplasia even though the animals have been subjected to these diets for 6 to 7 weeks. It may be of interest that the goldfish interrenal reacts promptly to injections of mammalian ACTH producing a picture which cannot easily be overlooked. The study of the goldfish interrenal will be published elsewhere. To these observations may be added the fact that our fish are in evident good health even after feeding on these high fat diets for many weeks. The findings indicate that our characteristic time-resistance curves are not due to "stress".

Hickman's (5) diagram for antioxidant activity may be used to explain the shape of the response curves under consideration assuming a "wanted reaction" limiting. To the "*in vivo*" and "*in vitro*" conditions Hickman adds the "*in transitu*" state. Metabolites and nutrients may be considered "*in transitu*" while journeying to and from the site of physiologic catalysis. It is not too unlikely that "accidental" oxidations ("unwanted reactions") will occur during this period—non-specific reactions which result in loss of metabolites. According to Hickman, biological antioxidants such as ascorbic acid (shown by Dugal and Fortier (2) to be important in cold resistance), nicotinic acid, pyridoxine, tocopherols, and tannins may play an important role in the body by conserving essential compounds while "*in transitu*". The non-specific oxidations decline with the addition of antioxidant material up to a certain concentration, after which these same antioxidants may start

to interfere with the wanted reactions—and, in addition, the curve for unwanted reactions rises. In a situation where either oxygen or a labile metabolite is limiting, as may well be the case in chill, an optimum antioxidant concentration will become obvious owing to maximum survival at that concentration. If this is to explain the characteristic time-resistance curves obtained, it must be assumed that continued feeding of phospholipid diets results in a progressively increasing physiological antioxidant titer in the body system responsible for thermal resistance. The phospholipid mixture used in many of the tests contained cephalin, which is a recognized source of antioxidant activity. It is possible that the cephalin content of some or all tissues was increased by feeding mixed phospholipids. Cholesterol, on the other hand, is certainly not an antioxidant, and it is very doubtful whether its similar effect on thermal resistance could have the same basis. It is emphasized that the antioxidant "explanation" of the time-resistance curves is a tentative working hypothesis of limited applicability.

Finally, an explanation of these characteristic curves may lie in complex changes of metabolic pattern induced by continued feeding of high lipid diets. Seasonal variations in thermal resistance, differences due to sex, the effects of thyroxine and thiourea (Hoar (6, Fig. 4) and unpublished) all indicate that changes in endocrinology modify the thermal resistance of goldfish. Endocrine regulation of fat metabolism may be seriously taxed by the high fat content of the diet, resulting in the changing pattern of resistance observed. This hypothesis remains to be investigated.

Acknowledgments

The work was supported by the National Research Council of Canada through grants-in-aid to W. S. Hoar and through two scholarships to D. G. Irvine. Contributions of pilchard oil were supplied by Dr. Sven Lassen of Van Camp Sea Foods, Terminal Island, California. Mr. J. Eichberg of the American Lecithin Company donated the granulestin. Dr. D. R. Idler of the Fisheries Experimental Station in Vancouver gave helpful advice on numerous occasions. It is a pleasure to acknowledge the assistance which made this study possible.

References

1. Bloor, W. R. and Snider, R. H. Phospholipid content and activity in muscle. J. Biol. Chem. **107**, 459–470 (1934).
2. Dugal, L. P. and Fortier, G. Ascorbic acid and acclimatization to cold in monkeys. J. Appl. Physiol. **5**, 143–146 (1952).
3. Fry, F. E. J. Effects of the environment on animal activity. Univ. Toronto Studies Biol. Ser. No. 55; Publ. Ont. Fisheries Research Lab. No. 68, 1–62 (1947).
4. Heilbrunn, L. V. An outline of general physiology. 3rd ed. W. B. Saunders Co., Philadelphia. 1952.
5. Hickman, K. C. D. Practical application of physiological antioxidants. *In* Biological antioxidants: transactions of the fourth conference. *Edited by* Mackenzie, C. G. Josiah Macy Jr. Foundation. 1949. pp. 114–123.
6. Hoar, W. S. Seasonal variations in the resistance of goldfish to temperature. Trans. Roy. Soc. Can. V, Ser. 3, **49**, 25–34 (1955).
7. Hoar, W. S. Photoperiodism and thermal resistance of goldfish. Nature, **178**, 364–365 (1956).

8. HOAR, W. S. and COTTLE, M. K. Dietary fat and temperature tolerance of goldfish. Can. J. Zool. **30**, 41–48 (1952).
9. HOAR, W. S. and COTTLE, M. K. Some effects of temperature acclimatization on the chemical constitution of goldfish tissues. Can. J. Zool. **30**, 49–54 (1952).
10. HOAR, W. S. and DORCHESTER, J. E. C. The effect of dietary fat on the heat tolerance of goldfish (*Carassius auratus*). Can. J. Research, D, **27**, 85–91 (1949).
11. MAYER, A. et SCHAEFFER, G. Recherches sur les constantes cellulaires teneur des cellules en eau. I. Discussion théorétique. L'eau, constante cellulaire. J. physiol. et pathol. gén. **16**, 1–16 (1914).
12. MAYER, A. et SCHAEFFER, G. Recherches sur les constantes cellulaires teneur des cellules en eau. II. Rapport entre la teneur des cellules en lipoïdes et leur teneur en eau. J. physiol. et pathol. gén. **16**, 23–38 (1914).
13. PRECHT, H., CHRISTOPHERSEN, J., and HENSEL, H. Temperatur und Leben. Springer-Verlag, Berlin. 1955.
14. SELYE, H. Textbook of endocrinology. Acta, Montreal. 1949. pp. 836–867.
15. SUNDSTROEM, E. S. The physiological effects of tropical climate. Physiol. Revs. **7**, 320–362 (1927).
16. WILBUR, C. G. and DEL POMO, M. Comparative study of lipids in whole carcasses of Arctic and non-Arctic fish. Proc. Soc. Exptl. Biol. Med. **72**, 418–420 (1949).

A SQUASH METHOD FOR SOMATIC CHROMOSOMES OF APHIDS[1]

LEO A. DIONNE[2] AND P. B. SPICER[2]

Abstract

Embryonic aphids are a good source of aphid chromosomes when extruded and left for 15 minutes in 25% Ringer's solution, fixed for 1 hour in a mixture of 2 parts glacial acetic acid, 2 parts 95% ethyl alcohol, and 0.125 parts of chloroform. The embryos are then hydrolyzed for 5 minutes in normal hydrochloric acid at 60° C. and stained in Gomori's hematoxylin for 30 minutes at 60° C. After differentiation in 45% acetic acid for ½ to 2 hours, the embryos are placed on a microscope slide, squashed under a cover glass, and mounted in 45% acetic acid. Individual plates are spread and flattened at low magnification with the point of a scalpel by tapping and pressing the cover glass over the figure. Unused embryos in 45% acetic acid and prepared slides will keep in "deep freeze" for about two weeks. Permanent preparations are made by floating the cover glasses from frozen slides in cold 95% ethyl alcohol, dehydrating in absolute alcohol, and mounting in an alcohol-based mounting medium.

Introduction

Until recently, most studies of aphid chromosomes were made from sectioned material. Colling (1) has reported on a squash method for the somatic chromosomes that apparently gives satisfactory results. This method involves osmic acid fixation and is reportedly most useful with permanent slides. Other squash techniques using acetocarmine, aceto-orcein, Feulgen, and Gomori's hematoxylin were not fully satisfactory for aphid chromosomes at the Fredericton laboratory. To fill this need, a consistently dependable squash method that does not require osmic acid fixation has been developed. The disadvantages of osmic acid are bypassed through the use of more manageable and commonly used reagents. The method has proved useful for detailed studies of aphid chromosomes without recourse to permanent slides. However, the method is well adapted to the making of permanent preparations should these be desired.

The following procedure has been used in a study of the somatic chromosomes of four species of aphids infesting potatoes, and for a number of species infesting other host plants.

Materials and Procedure

1. Extrude the embryos from about 25 adult aphids in a 25% Ringer's A solution (2). Remove and discard the adult bodies. Leave the embryos in the dilute Ringer's for 15 minutes.

2. Remove the Ringer's solution and replace it with a mixture of 2 parts glacial acetic acid, 2 parts ethyl alcohol, and 0.125 parts chloroform. Leave for 1 hour.

3. Hydrolyze the embryos (in a lens paper bag) in normal hydrochloric acid at 60° C. for 5 minutes.

[1]Manuscript received June 21, 1957.
Contribution No. 3652, Entomology Division, Science Service, Department of Agriculture, Ottawa, Canada.
[2]Field Crop Insect Section, Entomology Laboratory, Fredericton, N.B.

4. Wash briefly in distilled water and transfer to Gomori's hematoxylin (3) at 60° C. for 30 minutes.

5. Remove from the stain and place in 45% acetic acid for a minimum of 30 minutes.

6. Place five or six stained embryos on a clean microscope slide in a minimal amount of 45% acetic acid.

7. Apply a cover glass and gently squash the preparation by tapping it with a blunt instrument.

8. After an initial squashing, flood the area under the cover glass with 45% acetic acid, take up the excess acid with a piece of blotting paper, and press the slide between blotters. The slide should now be ready for examination. A good preparation shows numerous sharply stained nuclei spread out in a thin, even film.

Discussion

The embryos are extruded in a Syracuse dish by applying pressure on the abdomen of each aphid with a small glass rod. The prefixation solution is made by diluting a standard Ringer's A solution (Darlington and LaCour, (2, p. 118)) with distilled water in the ratio of 1:3. This treatment prevents a peculiar heteropycnotic condition of the nuclei in material not so treated (Figs. 1 and 2). It also results in contraction and better spreading of the chromosomes.

Changing the solutions is simplified if the liquid containing the embryos is taken up with a special pipette made by attaching a rubber bulb to one end of a 5-in. piece of glass tubing having a 5-mm. bore. The solution and aphids can thereby be transferred to a 19-mm. micro-Buchner funnel fitted with a disk of lens paper that is fluted to form a cup. The lens paper cup is then taken from the funnel and placed in a Syracuse dish where the fixing solution is carefully distributed around the periphery of the disk so as to cover the embryos with a thin film of fixative.

Before hydrolysis, it is advisable to place the lens paper disk carrying the embryos on a somewhat larger disk. The edges of both can then be drawn together with forceps so as to enclose the embryos in a small "bag". The "bag" can be kept closed by tying it with cotton thread. The free ends of the thread serve to fasten the "bag" to the end of a glass rod so as to prevent the material from floating during hydrolysis and staining.

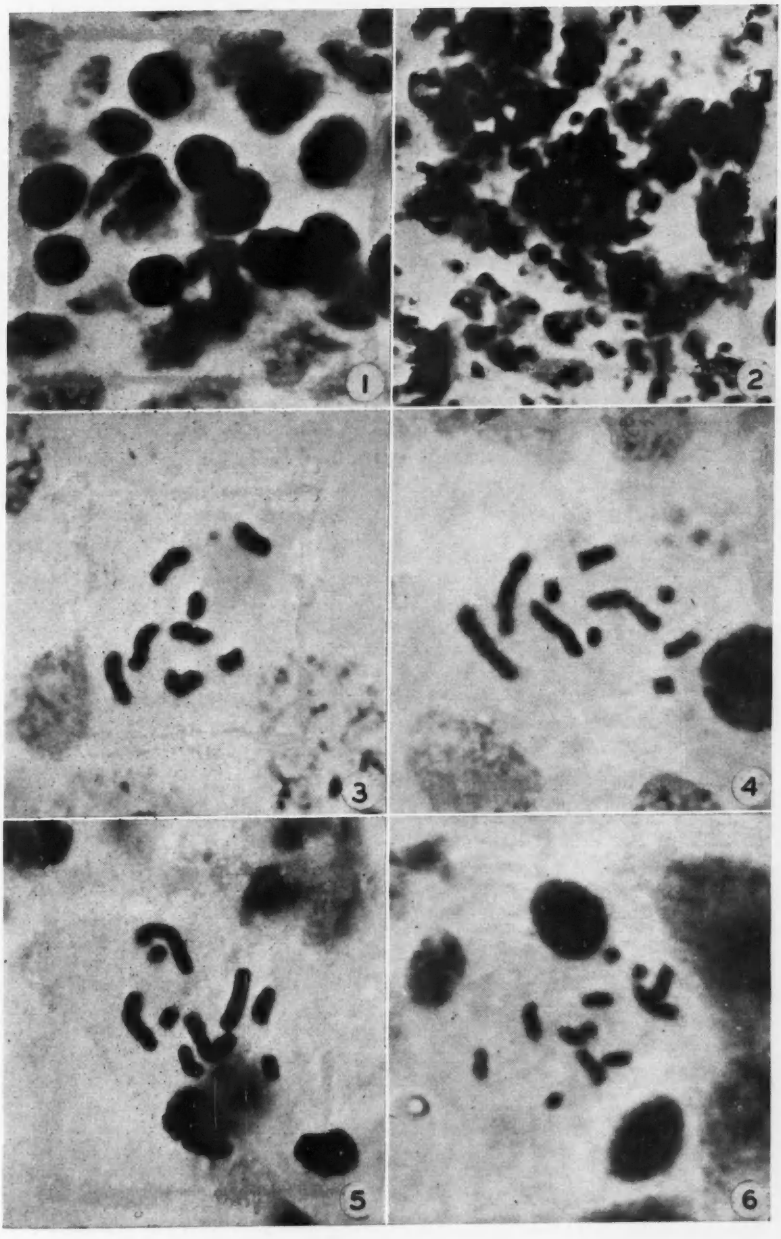
If it is desired to examine the embryos as soon as staining is completed, it is necessary to wash the "bag" of embryos in several changes of 45% acetic acid; otherwise the embryos are difficult or impossible to see in the very dark staining solution.

FIG. 1. Sharply stained nuclei from tissues treated with dilute Ringer's solution before fixation. × 2400

FIG. 2. Heteropycnotic aspect of nuclei in tissues fixed without pretreatment with dilute Ringer's solution. × 2400

FIGS. 3–6. Somatic chromosome plates of *Aphis abbreviata* Patch $2n=8$, *Macrosiphum solanifolii* (Ashm.) $2n=10$, *Aulacorthum solani* (Kltb.) $2n=10$, and *Myzus persicae* (Sulz.) $2n=12$ stained with Gomori's hematoxylin by the method given. × 2400

PLATE I



Dionne and Spicer—Can. J. Zool.

The chromosome plates are seldom perfectly spread out or flattened when first observed but most can be made usable by gentle tapping of the area over the plate with the point of a scalpel to spread the chromosomes, followed by somewhat greater pressure to flatten the plate. Spreading and flattening must, of course, be done at a lower power. Squashing of individual plates can only be done if just the right amount of 45% acetic acid is under the cover slip. Since there is a tendency for the acetic acid to dry out, it must be replaced from time to time by a small drop placed on one edge of the cover glass. If an excess is added, the slide should be removed from the microscope stage and gently blotted; otherwise the plates float away when manipulated. However, once a plate has been well flattened, it is rarely displaced.

At the Fredericton laboratory, all good plates are photographed as soon as observed and all chromosome measurements are made from ×4 enlargements of these photographs. A temporary slide lasts for about two weeks if kept in "deep freeze". All unmounted material may also be kept for some time by the same method. Permanent preparations may be made by floating off the cover glasses from frozen slides in 95% alcohol and mounting the slide and cover slip separately in Gurr's neutral mounting medium.*

Figs. 3–6 indicate the effectiveness of this technique for the chromosomes of four species of potato-infesting aphids.

*George T. Gurr Ltd., London, S.W. 6, England.

Acknowledgment

Grateful acknowledgment is made for the technical help of Mr. D. W. Rideout, formerly of the Fredericton laboratory, now on the staff of the Sault Ste. Marie High School, Sault Ste. Marie, Ont., Canada.

References

1. COLLING, A. W. Aphid chromosomes. Nature, **176**, 207–208 (1955).
2. DARLINGTON, C. D. and LACOUR, L. F. The handling of chromosomes. George Allen & Unwin, Ltd., London. 1947.
3. MELANDER, Y. and WINGSTRAND, K. G. Gomori's hematoxylin as a chromosome stain. Stain Technol. **28**, 217–223 (1953).

COCCIDIA (PROTOZOA: EIMERIIDAE) OF NORTH AMERICAN WILD GEESE AND SWANS[1]

HAROLD C. HANSON, NORMAN D. LEVINE, AND VIRGINIA IVENS

Abstract

In a survey of the coccidia of 362 wild geese and swans from Canada and the United States, including subspecies and populations of *Anser albifrons*, *A. caerulescens*, *A. rossi*, *Branta canadensis*, *B. bernicla*, and *Olor columbianus*, the following coccidia were found: *Eimeria anseris*, *E. brantae*, *E. fulva*, *E. hermani*, *E. magnalabia* (syn., *E. striata*), *E. nocens*, *E. truncata*, *E. clarkei* n. sp., *E. farri* n. sp., and *Tyzzeria anseris*. Reports in the literature on the species of coccidia occurring in wild and domestic geese and their prevalence and geographic distribution are summarized with particular reference to North American flyways.

Introduction

The increasingly intensive use of submarginal land for agriculture and other purposes has tended to concentrate waterfowl populations in winter on federal, state, and private refuges. This trend toward concentration of existing populations has been particularly true for wild geese, many populations on the continent now being largely confined in winter to refuge areas. These concentrations produce at least the potential hazard of widespread losses from epizootics of one kind or another. Thus the problem of refuge sanitation adds another burden to the responsibilities of the refuge manager.

Although wild geese appear to be relatively free from serious epizootics, the occurrence of an outbreak of renal coccidiosis in Canada geese at the Pea Island National Wildlife Refuge, North Carolina (Critcher (6)) and later the recognition of the gizzard worm, *Amidostomum anseris*, as an important cause of winter losses among the Canada geese at this refuge (Herman and Wehr (22), Cowan and Herman (5)) indicates the possibility of similar outbreaks elsewhere. The potential danger of disease outbreaks is illustrated by the fact that the area around Horseshoe Lake State Game Refuge in southern Illinois alone winters about half of the Mississippi flyway population and, together with the Union County State Wildlife Refuge and the Crab Orchard National Wildlife Refuge in southern Illinois, winters about 90 per cent of the flyway population.

Since 1949 a cooperative study has been carried out by the Illinois Natural History Survey and the University of Illinois College of Veterinary Medicine on parasites of wild geese in the United States and Canada, with special reference to the Mississippi flyway population of Canada geese. The coccidia were first reported on by Levine (26), the blood parasites by Levine and Hanson (29), and the filariae by Hanson, Levine, and Kantor (17) and Hanson (15).

[1] Manuscript received May 24, 1957.
Contribution from the Illinois Natural History Survey, Urbana, and College of Veterinary Medicine and Agricultural Experiment Station, University of Illinois, Urbana, Illinois, U.S.A.

The present paper reports the results of surveys carried out in 1954, 1955, and 1956, describes two new species of *Eimeria*, redescribes two more, gives additional details on the morphology of other species, and summarizes available information on the host–parasite relationships, prevalence, and geographic distribution of the coccidia of geese.

Materials and Methods

The 362 fecal and intestinal content samples examined in the present study were obtained from 11 species and subspecies of wild geese and swans.

The bird nomenclature followed in this paper is that of Delacour and Mayr (9), Delacour (7, 8), and of an anonymous author (2). We have been dependent for subspecific determinations of many of the hosts on the persons who made the collections in the field. While it may at first seem questionable to rely on field identifications of subspecies, the differences in size between most of them as well as differences in their ranges permit the use of subspecific names with confidence. For example, it may be difficult to differentiate some individuals in a mixed series of museum skins of *B. canadensis hutchinsi* and *B. c. minima*. However, the former breeds in the eastern Arctic and winters in the Central flyway, while the latter breeds in Alaska and winters in California, so that the probability of either one being taken in the range of the other is extremely small. The exact identity of the somewhat larger "lesser Canada goose", which winters on the Pacific coast, is open to question, but on the basis of known range it is more likely *B. c. taverneri*, a subspecies which breeds along the north and northwestern coast of Alaska (Delacour (7)) rather than *B. c. parvipes*, which breeds mainly in the Arctic portions of the Canadian mainland west of Hudson Bay.

Fecal or intestinal content samples were obtained by various collectors (see Acknowledgments for names) in several ways: from feeding grounds where only a single known species of goose had been feeding just prior to collection; from geese bagged by Indian hunters in northern Canada or by American hunters in the United States; from geese trapped with cannon nets; and from flightless young and adult Canada geese captured in northern Ontario and Manitoba with drive traps.

All fecal samples were preserved in 2.5% potassium bichromate solution for transportation or shipment to Urbana. In the laboratory each sample was mixed thoroughly, placed in a thin layer in a Petri dish, and allowed to incubate at room temperature for 5 to 7 days. If the oocysts had not sporulated by that time, they were allowed to incubate longer. The coccidia were concentrated for examination by centrifugal flotation in Sheather's sugar solution.

Results

The findings are given in Table I and discussed below for each coccidian species. Table I also summarizes information from the literature on the prevalence of coccidia in geese and swans.

TABLE I

PREVALENCE OF COCCIDIA IN GEESE AND SWANS (%)

Host species and locality	No. birds studied	*Eimeria anseris*	*Eimeria brantae*	*Eimeria clarkei*	*Eimeria farri*	*Eimeria fulva*	*Eimeria hermani*	*Eimeria magnalabia*	*Eimeria nocens*	*Eimeria parvula*	*Eimeria truncata*	*Tyzzeria anseris*	Reference
Olor columbianus (whistling swan)													
Chesapeake Bay, Md.	13											15	This paper
Tule Lake Wildlife Refuge, Calif.	10												This paper
Anser anser (domestic goose)													
Europe		+							+	+	+		Kotlán (24)†
Europe												+	Nieschulz (33)
United States											+		Various authors‡
Maryland	2											100	Farr and Wehr (14)
United States						Expt.*	Expt.*	Expt.*				Expt.*	Farr (11, 12)
Anser albifrons frontalis (white-fronted goose)													
Trimble Wildlife Area, Mo.	9							44				56	This paper
Tule Lake Wildlife Refuge, Calif.	13											15	This paper
Sacramento Wildlife Refuge, Calif.	6				17							33	This paper
Merced Wildlife Refuge, Calif.	2												This paper
Anser caerulescens caerulescens (blue and lesser snow geese)													
Ft. Severn and Weenusk, Ont.	73	4		1		3	12	7	5			8	This paper
Tule Lake Wildlife Refuge, Calif.	14							7				57	This paper
Sacramento Wildlife Refuge, Calif.	12												This paper
Merced Wildlife Refuge, Calif.	1												This paper
Anser caerulescens atlanticus (greater snow goose)													
Pea Island, N. Carolina	8											75	Farr (11)
Cap Tourmente, Quebec	28											4	This paper
Anser rossi (Ross's goose)													
Perry River, N.W.T., Canada	6												Levine (28)
Tule Lake Wildlife Refuge, Calif.	4											50	This paper
Merced Wildlife Refuge, Calif.	3										33	33	This paper
Branta canadensis interior (Todd's Canada goose)													
Pea Island, N. Carolina											+		Critcher (6)
Pea Island, N. Carolina	Several hundred											>50	Farr (11)
Pea Island, N. Carolina						+	+	+					Farr (12)
Pea Island, N. Carolina	450										34		Farr (13)
Chesapeake Bay, Md.	43							5			9	47	This paper
Seneca Falls, N.Y.						+							Farr (12)

TABLE I—*Concluded*

PREVALENCE OF COCCIDIA IN GEESE AND SWANS (%)—*Concluded*

Host species and locality	No. birds studied	*Eimeria anseris*	*Eimeria brantae*	*Eimeria clarkei*	*Eimeria farri*	*Eimeria fulva*	*Eimeria hermani*	*Eimeria magnalabia*	*Eimeria nocens*	*Eimeria parvula*	*Eimeria truncata*	*Tyzzeria anseris*	Reference
Seney Wildlife Refuge, Mich.					.	+	+	+				+	Farr (11, 12) Wehr and Herman (35)
Horseshoe Lake, Ill. (Nov.–Dec. 1950)	48							10				67	Levine (27)
Horseshoe Lake (Nov.–Dec. 1954)	48							2				10	This paper
Horseshoe Lake (Feb. 1955)	40											8	This paper
Ft. Severn, Ont. (goslings)	17							24				18	This paper
Owl River, Man. (goslings)	7							14				43	This paper
Branta canadensis moffitti (Moffitt's Canada goose)													
Malheur Wildlife Refuge, Ore.	5											20	This paper
Tule Lake Wildlife Refuge, Calif.	16										13	38	This paper
Branta canadensis taverneri (?) (Taverner's Canada goose)													
Sacramento Wildlife Refuge, Calif.	1											100	This paper
Branta canadensis parvipes (lesser Canada goose)													
Perry River, N.W.T., Canada	4		25										Levine (28)
Branta canadensis hutchinsi (Richardson's Canada goose)													
York Factory, Man.	6	33	17					50					This paper
Branta canadensis minima (cackling Canada goose)													
Lower Klamath Lake Wildlife Refuge, Ore.	14						14	21				7	This paper
Sacramento Wildlife Refuge, Calif.	7							14				43	This paper
Merced Wildlife Refuge, Calif.	5												This paper
Branta bernicla hrota (Atlantic brant)													
Isle aux Pommes, Que.	8											25	This paper

* From *Branta canadensis interior*.
†And various other authors for *E. truncata*.
‡ See Farr and Wehr (1952) for references.

Eimeria anseris Kotlán, 1932 (23), emend. Kotlán, 1933 (24) (Fig. 1)

This species was found in fecal samples from *Anser c. caerulescens* and *Branta c. hutchinsi* from York Factory, Manitoba. These are both new host records. *E. anseris* has apparently not been previously reported from the western hemisphere.

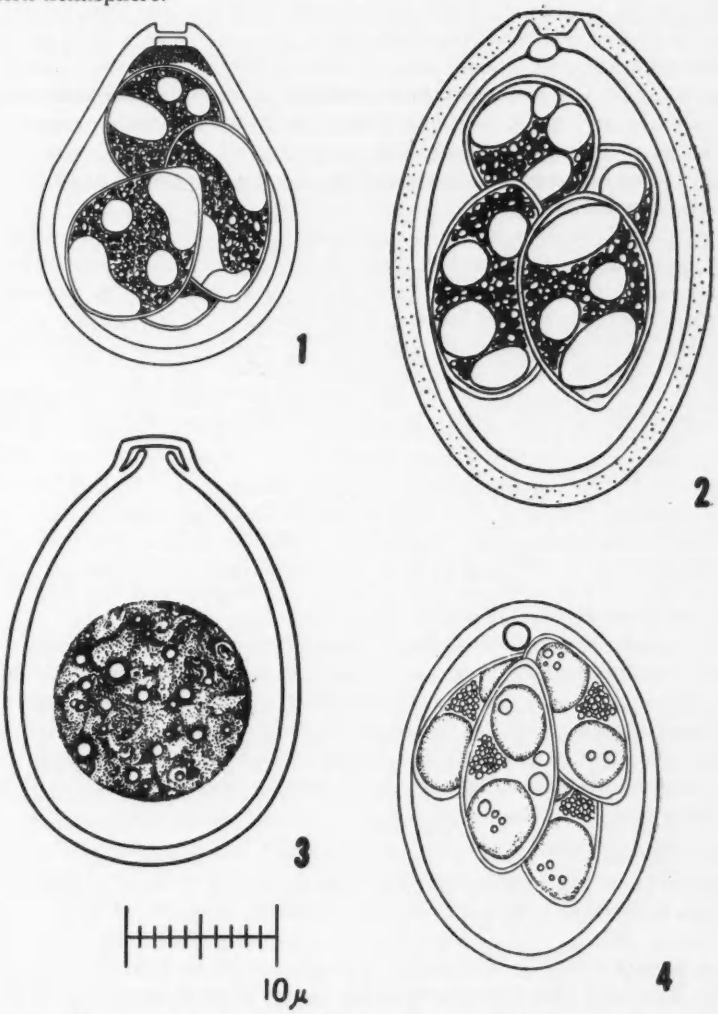


FIG. 1. *Eimeria anseris* Kotlán, 1932. Sporulated oocyst from snow goose, *Anser caerulescens caerulescens*. × 2300

FIG. 2. *Eimeria nocens* Kotlán, 1933. Sporulated oocyst from snow goose, *Anser caerulescens caerulescens*. × 2300

FIG. 3. *Eimeria clarkei* n. sp. Oocyst from snow goose, *Anser caerulescens caerulescens*. × 2300

FIG. 4. *Eimeria farri* n. sp. Sporulated oocyst from white-fronted goose, *Anser albifrons frontalis*. × 2300

E. anseris was originally described from the domestic goose, *Anser anser*, in Europe. Since Kotlán's (24) description is incomplete, the sporulated oocysts are redescribed here.

Description

The oocyst might be called pear-shaped, although its form is actually closer to that of a sphere surmounted by a truncate cone, with a micropyle at the truncate end. Some oocysts are rather squat, others somewhat elongate, and a few somewhat asymmetrical. Forty-four sporulated oocysts from a single snow goose measured 16 to 19 by 20 to 24 μ, with a mean of 17.2 by 21.7 μ. Their length–width ratios ranged from 1.1 to 1.4, with a mean of 1.27. These figures may be compared with the range of 13 to 18 by 16 to 23 μ given by Kotlán (24).

Oocyst wall smooth, colorless, composed of a single layer about 1 μ thick, wall slightly thickened around micropyle, but incised sharply to form a plate or shelf across the micropyle itself. Just beneath the micropyle of the sporulated oocysts and forming a seal beneath it is a mass of amorphous material. This is the oocyst residuum; it was not seen in unsporulated oocysts. There is no refractile granule.

Sporocysts almost completely fill the oocyst. Sporocysts ovoid, with a wall slightly thickened at the small end, but without a true Stieda body. Sporocysts approximately 7 to 9 by 10 to 12 μ, some being more elongate than others. Two sporozoites and a large residuum almost completely fill each sporocyst. Kotlán (24) stated that the sporocyst residuum was small, but this is a relatively minor difference. The sporozoites often lie more or less transversely at the anterior and posterior ends of the sporocyst.

Eimeria nocens Kotlán, 1933 (24) (Fig. 2)

This species was found in *Anser c. caerulescens* (new host record). This species has apparently not been reported from the western hemisphere before.

E. nocens was originally described from the domestic goose, *Anser anser* in Europe. Kotlán (24) did not describe the sporulated oocysts, but gave a photomicrograph of one in which details cannot be clearly discerned. The following description of the sporulated oocysts from our material therefore provides new information on this stage.

Description

Oocysts ovoid but flattened at the micropylar end. Twenty-one sporulated oocysts from three snow geese measured 29 to 33 by 19 to 24 μ, with a mean of 31.0 by 21.6 μ. Their length–width ratios ranged from 1.3 to 1.7, with a mean of 1.45. Oocyst wall smooth, composed of two layers, the outer one 1.3 μ thick and pale yellow, the inner one 0.9 μ thick and almost colorless. Prominent micropyle present. True micropylar cap absent, but the micropyle appears to be present only in the inner wall and is covered by the outer wall. Neither oocyst residuum nor refractile granule present. However, part of the oocyst wall often forms one or more roundish protuberances just below the micropyle.

Sporocysts broadly ellipsoidal, with a thin wall. A very small Stieda body is present in some, but none can be seen in others. Fourteen sporocysts measured 10 to 14 by 8 to 10 μ, with a mean of 12.1 by 9.1 μ. The two sporozoites usually lie head to tail in the sporocysts and contain two or more large, clear globules which almost obscure their outline. There is a large amount of residual material in the sporocysts; it does not form a circumscribed body, but fills the space between the sporozoites.

Eimeria magnalabia Levine, 1951 (26)

Synonym: *Eimeria striata* Farr, 1953 (12)

This species was found in *Anser albifrons frontalis* (new host record), *A. c. caerulescens* (new host record), *B. canadensis interior*, *B. c. hutchinsi* (new host subspecific record), and *B. c. minima* (new host subspecific record).

Thirty-one sporulated oocysts from five *A. c. caerulescens* measured 16 to 19 by 20 to 25 μ, with a mean of 17.5 by 22.3 μ. Their length–width ratios ranged from 1.1 to 1.4, with a mean of 1.28.

This species was originally described by Levine (26, 27) from *Branta c. interior* from Horseshoe Lake Game Refuge in southern Illinois. Farr (12) described it under the name *Eimeria striata* from *B. canadensis* in North Carolina and Michigan, and also transmitted it experimentally to the domestic goose.

Farr (12) stated that *E. striata* differed from *E. magnalabia* in having one or more polar bodies and a small sporocyst Stieda body, and in the thickness of the two layers of the oocyst wall at the micropylar end. However, we have found by study of a larger series of *E. magnalabia* than was available when the species was first described that there is no significant difference between the two forms. In this study oocysts were examined not only from *Anser caerulescens*, *A. albifrons*, and *Branta c. hutchinsi* but also from the type host, *Branta canadensis interior*, from the type locality, Horseshoe Lake, Illinois.

E. magnalabia has no polar refractile granules; the structures figured by Farr are not the colorless granules produced during sporogony which this name designates. Farr's structures are yellowish in color, not sharply refractile, and are actually derived from the oocyst wall, from which they hang down around the micropyle. The rounded granules in Farr's drawing are more discreet than the thickenings around the micropyle illustrated by Levine (27), but all intermediate stages between these two shapes have been seen. Actually, the thickenings illustrated by Levine are more bulbous and regular than usual.

When seen under a microscope with achromatic objectives, some of the sporocysts appeared to have a small Stieda body while others did not. When studied carefully under a microscope with apochromatic objectives, this structure was seen not to be a thickened knob formed by the sporocyst wall but a small mass of clear material just beneath the wall. This is what was illustrated by Farr, except that the line demarcating it from the sporocyst wall was not indicated. It was not illustrated by Levine.

The third criterion used by Farr to differentiate *E. striata* from *E. magnalabia* was the morphology of the oocyst wall. In both species the wall was described as composed of two layers, the outer one being yellowish, thick, finely striated and pitted, and the inner one being thin and colorless. However, Levine stated that the inner wall did not extend all the way to the micropyle, while Farr stated that it did, actually becoming thicker around the micropyle. In the present study it was observed that in some oocysts the inner wall conformed to Levine's description, while in others it extended to the micropyle.

Eimeria brantae Levine, 1953 (28)

This species was found in a fecal sample from *Branta canadensis hutchinsi*. This is a new subspecific host record.

E. brantae was originally described from *Branta canadensis parvipes* from the mouth of the Perry River on the Arctic Ocean in the District of MacKenzie, Northwest Territories. Only a single sporulated oocyst was seen, and a detailed morphological study was not made of it, the description being based on unsporulated oocysts. The oocysts from *B. c. hutchinsi* again failed to sporulate, so no additional morphological information can be given.

When she named *E. hermani* (see below), Farr (12) remarked on its resemblance to *E. brantae*, but considered the differences sufficient to justify establishing her species as new. We concur in this. *E. hermani* is not truly ovoid but ellipsoidal, with slightly flattened sides, while *E. brantae* is ovoid. In addition, while the inner oocyst wall of *E. brantae* is thickened around the micropyle, the thickening is smooth and does not resemble the prominent, irregularly thickened wall around the micropyle of *E. hermani*.

Eimeria fulva Farr, 1953 (12)

This species was found in *Anser c. caerulescens*. This is a new host record.

E. fulva was originally described from the Canada goose, *Branta canadensis*, from North Carolina, New York, and Michigan. Farr (12) also transmitted it to the domestic goose.

Eimeria hermani Farr, 1953 (12)

This species was found in fecal samples from *Anser c. caerulescens* (new host record) and *Branta c. minima* (new host subspecific record).

Thirty-eight oocysts from two snow geese measured 24 to 27 by 16 to 19 μ, with a mean of 25.3 by 17.8 μ. Their length–width ratios ranged from 1.3 to 1.6, with a mean of 1.42.

E. hermani was originally described from the Canada goose, *Branta canadensis*, from North Carolina and Michigan. Farr (12) also transmitted it experimentally to the domestic goose.

Eimeria ***clarkei*** n. sp. (Fig. 3)

This species was found in a fecal sample from a snow goose, *Anser caerulescens caerulescens*, from Weenusk, Ontario. No completely sporulated oocysts were found, even after repeated incubation at room temperature in

a thin layer of 2.5% potassium bichromate solution. However, a number of oocysts containing four sporoblasts were seen, so that this species can be safely assigned to the genus *Eimeria*.

Description

The oocysts resemble a round-bottomed flask with a short, narrow neck. Eighteen oocysts measured 25 to 30 by 18 to 21 μ, with a mean of 27.3 by 19.3 μ. Their length–width ratios ranged from 1.3 to 1.5, with a mean of 1.42. The oocyst wall is colorless, smooth, and composed of a single layer 1 μ thick. There is a prominent micropyle at the protruding anterior end. The structure around it is unusual. The oocyst wall divides into two layers separated by a little space. The inner layer forms the wall of the micropyle, while the outer layer forms a flattened cover over it. More often than not, the inner layer does not quite touch the outer one anteriorly. The oocyst wall is not delicate, and does not shrink readily in the Sheather's sugar solution used for flotation. The sporont in the unsporulated oocysts is relatively small, ranging from 9 to 13 μ in diameter, with a mean of 12 μ. The partially sporulated oocysts containing four sporoblasts have no oocyst residuum or refractile granule.

E. clarkei resembles *E. anseris* in the general appearance of its oocyst wall, but is considerably larger, lacks an oocyst residuum, and differs markedly in the structure of its micropyle.

E. clarkei also resembles *E. truncata* somewhat, but a comparison with Kotlán's (24) and Lerche's (25) descriptions of the latter shows that it is quite different. Kotlán made a point of the resemblance between *E. truncata* and *E. nocens*, stating that aside from the unmistakable difference in size and a difference in configuration of the micropyle wall observable on careful inspection, the oocysts of the two species are quite similar. *E. clarkei*, on the other hand, bears little resemblance to *E. nocens*. *E. clarkei* is larger than *E. truncata*. Kotlán (24) emphasized that he had never encountered an oocyst of the latter longer than 24 μ and that oocysts 22 μ long seldom occurred. The oocysts measured by Lerche (25) were 14.3 to 22.8 μ long and 11.7 to 15.6 μ wide. The oocysts of *E. clarkei* are 25 to 30 μ long. Kotlán did not indicate the number of layers in the oocyst wall of *E. truncata*, but *E. nocens*, which he said it resembled, has two. Lerche (25) stated that the wall has a double contour, but illustrated only one layer. *E. clarkei* has a single layer. Kotlán mentioned that the wall of *E. truncata* is delicate, shrinking very quickly during the concentration process; this was not true of *E. clarkei*. Finally and most important, the structure of the micropyle of *E. clarkei* differs from those described for all other coccidian species from birds of the order Anseriformes. Despite the fact that no completely sporulated oocysts were seen, the establishment of a new species for it appears justified. This species is named in honor of Dr. C. H. D. Clarke of the Ontario Department of Lands and Forests.

Eimeria ***farri*** n. sp. (Fig. 4)

This species was found in a fecal sample from one of six white-fronted geese, *Anser albifrons frontalis*, at the Colusa Wildlife Refuge, Sacramento, California.

Description

Oocysts ellipsoidal, slightly ovoid, sometimes slightly asymmetrical. Micropyle absent. Oocyst wall smooth, colorless to very pale yellow, composed of a single layer about 0.9 μ thick. Oocyst polar granule present. Oocyst residuum absent. Sporocysts elongate-ovoid, 12 to 13 by 6 μ, with a small Stieda body. Sporozoites at ends of sporocysts. Sporocyst residuum finely granular; in addition to it there are a number of more or less scattered coarse granules. Only three sporulated oocysts were measured, although more were seen. They ranged from 22 to 23 by 17 to 20 μ, with a mean of 22.8 by 18.0 μ. Their length–width ratios ranged from 1.2 to 1.3, with a mean of 1.27.

The oocysts of *E. farri* differ from those of all other species of *Eimeria* from geese except *E. parvula* Kotlán, 1933 (24) in lacking a micropyle. However, the oocysts of *E. parvula* measure only 10 to 15 by 10 to 14 μ and are spherical to subspherical, while the oocysts of *E. farri* are much larger and more elongate. Since the sporulated oocysts of *E. parvula* have not been described, these cannot be compared.

Tyzzeria anseris Nieschulz, 1947 (33)

In the present survey, *Tyzzeria* was found in fecal samples from *Olor columbianus* (new host record), *Anser albifrons frontalis* (new host record), *Anser caerulescens caerulescens* (new host subspecific record), *Anser rossi* (new host record), *Branta canadensis interior*, *Branta c. moffitti* (new host subspecific record), *B. c. taverneri* (?) (new host subspecific record), *B. c. minima* (new host subspecific record), and *B. bernicla hrota* (new host record).

The correct specific name for the *Tyzzeria* of geese has been discussed by Levine (27) and Farr and Wehr (14). Three species of *Tyzzeria* have been named from birds. The type species, *T. perniciosa* Allen, 1936 (1) was described from the domestic Pekin duck, *Anas boschas* (syn., *A. domesticus*) on Long Island, New York. *T. alleni* Chakravarty and Basu, 1946 (3) was described from the cotton teal, *Cheniscus* (syn., *Nettapus*) *coromandelianus*, in India. *T. anseris* Nieschulz, 1947 (33) was described from the domestic goose, *Anser anser*, in the Netherlands. Since the oocysts of all three forms are morphologically alike, proof of their specific distinctness must depend on differences in their parasitic, intracellular stages or in their host specificity. However, no studies have been made of the intracellular stages of *T. alleni* or *T. anseris*, while the only cross-transmission studies are those of Nieschulz (33), who failed to infect a single duck with sporulated oocysts of *T. anseris* from *Anser anser*, and of Farr (11), who infected *A. anser* with *Tyzzeria* sp. from *Branta canadensis*. Further cross-transmission studies are needed to determine whether *T. alleni* and *T. anseris* are synonyms of *T. perniciosa*. In the meantime, it is considered best to use the name *T. anseris* for the *Tyzzeria* of geese and swans.

Discussion

Available information on the species of coccidia reported from wild and domestic geese and swans and on their prevalence and geographic distribution is summarized in Table I.

Five species of coccidia, *Eimeria anseris*, *E. nocens*, *E. parvula*, *E. truncata*, and *Tyzzeria anseris*, have been found in the domestic goose. All these (except *E. parvula*) plus six others have been found in wild geese. *T. anseris* has also been found in the whistling swan, but no coccidia have apparently been reported from domestic swans. Furthermore, four of the species from wild geese (*E. fulva*, *E. hermani*, *E. magnalabia*, and *T. anseris*) were transmitted experimentally from *Branta canadensis* to the domestic goose by Farr (11, 12).

All of the 10 species of *Eimeria* described from geese except *E. parvula* have been found in North America, and four have been found in Europe. These last, *E. anseris*, *E. nocens*, *E. parvula*, and *E. truncata*, were all originally described from the domestic goose. The only one of these found so far in the domestic goose in North America is *E. truncata*. *E. anseris* and *E. nocens* were reported for the first time in North America in the present paper, but they were found in wild geese. *Tyzzeria anseris* has been found in domestic geese in Europe and in domestic and wild geese and the whistling swan in North America.

It is hardly necessary to point out that wild geese are a potential source of coccidial infection for domestic geese.

So far as wild geese alone are concerned, coccidia have been found in three of the four wild North American species of *Anser* and in both of the two North American species of *Branta*. Coccidia have not yet been reported from the Emperor goose, *A. canagica*, but this species has not yet been examined.

The most widely distributed species is *Tyzzeria anseris*, which has been found in all six goose species from which coccidia have been reported and in the whistling swan. *Eimeria magnalabia* has been found in three species of wild geese and has been transmitted experimentally to the domestic goose. *E. anseris* and *E. truncata* have been found in the domestic goose and two wild species. *E. fulva* and *E. hermani* have been found in two species of wild geese and have been transmitted experimentally to the domestic goose. *E. nocens* has been found in the domestic goose and one wild species. *E. brantae*, *E. clarkei*, and *E. farri* have been found in one wild species each.

While most species of *Eimeria* are generally considered to be confined to single host species or at least to single host genera, it is apparent that many of the goose *Eimeria* have a broader host spectrum. *E. anseris*, *E. fulva*, *E. hermani*, *E. magnalabia*, and *E. truncata* occur naturally in both *Anser* and *Branta*. So far, *E. clarkei*, *E. nocens*, *E. parvula*, and *E. farri* have been found only in *Anser*, and *E. brantae* only in *Branta*. Perhaps future study will reveal that some of these have a wider host range.

The continent has been divided for waterfowl into a series of flyways on the basis of their flight habits. Generally speaking, wild geese breed in the northern part of the continent and winter in the south. Their flyways are much more restricted than are those of ducks. There is relatively little crossing over between flyways, the birds in each forming largely self-contained populations. As a result, subspecific differences, and even specific ones, have arisen. For example, the subspecies of the Canada goose, *Branta canadensis*, which are successively encountered in proceeding westward from the north Atlantic coast are (roughly speaking) *canadensis*, *interior*, *hutchinsi*, *parvipes*, *moffitti*, *taverneri*, and *minima*. Some of these subspecies, notably *interior* and *hutchinsi*, are found in more than one adjacent flyway.

The flyways, at least as they apply to the goose populations whose coccidia have been studied, are:

(*1*) *The North Atlantic flyway.*—The greater snow geese of this flyway (*Anser c. atlanticus*) breed on Baffin and Ellesmere Islands and winter along the Atlantic coast from Chesapeake Bay to North Carolina. The Canada geese of this flyway (*B. c. canadensis*) (the coccidia of which have not yet been studied) breed in Labrador, Nova Scotia, and Newfoundland, and winter along the Atlantic coast from southern Nova Scotia to New Jersey. The Atlantic brant (*B. bernicla hrota*) of this flyway breed in the eastern Canadian Arctic and winter on the Altantic coast from New Jersey to North Carolina.

(*2*) *The South Atlantic flyway.*—The Canada geese of this flyway (*B. c. interior*) breed east of Hudson Bay and James Bay and on the Belcher Islands and winter along the Atlantic coastal region from Chesapeake Bay to North Carolina.

(*3*) *The Mississippi flyway.*—The Canada geese of this flyway (*B. c. interior*) breed in the District of Patricia of northern Ontario (which lies south of Hudson Bay and west of James Bay) and winter in the upper Mississippi Valley.

(*4*) *The Eastern Prairie flyway.*—The lesser snow geese of this flyway (*A. c. caerulescens*) breed on Southampton Island and in the District of Keewatin, Northwest Territories, and winter along the Gulf Coast of western Louisiana and in Texas. The Todd's Canada geese (*B. c. interior*) of this flyway breed in the muskeg of northern Manitoba and winter from east-central Arkansas to the Gulf Coast of western Louisiana and Texas. The Richardson's Canada geese (*B. c. hutchinsi*) of this flyway breed on southwestern Baffin Island, Southampton Island, and the shore of the Arctic Ocean from Melville Peninsula to Boothia Peninsula, and winter principally along the northwestern portions of the Gulf of Mexico.

(*5*) *The Central flyway.*—The white-fronted geese (*A. a. frontalis*) and lesser Canada geese (*B. c. parvipes*) of this flyway breed in the districts of Keewatin and eastern Mackenzie, Northwest Territories, and winter in southern United States west of the Mississippi River and south into Mexico.

(*6*) *The Pacific flyway.*—All the geese of this flyway in the present study except *B. c. moffitti* breed in the Arctic areas of western Canada and Alaska, and winter in California and Oregon; the *B. c. moffitti* were resident in the

refuges where their fecal samples were collected. For further details regarding each species see Cooch (4), Delacour (8), Hanson and Griffith (16), Hanson and Smith (19), Hellmayr and Conover (21), Lewis (31), and Manning (32).

The whistling swan breeds in arctic Alaska and Canada east to Baffin Island. The division line on the breeding grounds between the swans that winter on the Atlantic and Pacific coasts is not known. It is likely, however, that the swans which breed on Southampton Island and Baffin Island winter on the Atlantic coast.

The incidence of coccidia in the various flyways is summarized in Table II. Although our information is far from complete and will undoubtedly be changed as more data are accumulated, a study of the distribution of coccidia reveals some noteworthy relationships.

Tyzzeria anseris is again the most widespread coccidium, being found in all six flyways. *Eimeria magnalabia* has been found in all but the North Atlantic flyway. *E. hermani* is the next most frequent, having been found in all but the North Atlantic and Central flyways. *E. fulva* has been found in three flyways, the South Atlantic, Mississippi, and Eastern Prairie. *E. truncata* has been found only in the South Atlantic and Pacific flyways. *E. brantae* has been found only in the Eastern Prairie and Central flyways. The other four species have been found in a single flyway each, *E. farri* in the Pacific flyway, and *E. clarkei*, *E. anseris*, and *E. nocens* in the Eastern Prairie flyway. By a coincidence, the last two are the only species of *Eimeria* other than *E. truncata* and *E. parvula* which have been reported from Europe.

The distribution of *E. truncata* is particularly striking. This species, which causes a pathogenic kidney infection, has been found in domestic geese in many parts of North America, including Illinois (Levine, Morrill, and Schmittle (30), Farr and Wehr (14)), but among wild geese it has been reported only from the South Atlantic and Pacific flyways, and not from the flyways between. This does not seem due to the examination of too few birds. We ourselves failed to find it in 258 geese from the interior flyways, although we recognized it in four out of 43 Canada geese from Maryland and in three out of 103 geese from the West Coast.

This difference in distribution of *E. truncata* becomes even more striking when it is considered that it involves two different populations of the same subspecies of Canada goose, *B. c. interior*. Those of the South Atlantic flyway, which breed on the Belcher Islands and east of Hudson Bay and James Bay and winter at Pea Island, North Carolina, and other areas of the Middle Atlantic coast (Hanson and Griffith (16)), have a high infection rate (Table I), but those of the Mississippi flyway, which breed west of Hudson Bay and James Bay and winter in southern Illinois (Hanson and Smith (19)), are uninfected.

Of the 10 species of coccidia reported from North American wild geese, the greatest number (eight) was found in the Eastern Prairie flyway, while five were found in the South Atlantic and Pacific flyways, four in the Mississippi flyway, three in the Central flyway, and one in the North Atlantic flyway.

TABLE II

INCIDENCE OF COCCIDIA IN NORTH AMERICAN WILD GEESE AND SWANS BY FLYWAYS*

Flyway and host	*Eimeria anseris*	*Eimeria brantae*	*Eimeria clarkei*	*Eimeria farri*	*Eimeria fulva*	*Eimeria hermani*	*Eimeria magnalabia*	*Eimeria nocens*	*Eimeria truncata*	*Tyzzeria anseris*
North Atlantic										
Olor columbianus										+
Anser caerulescens atlanticus										+
Branta bernicla hrota										+
South Atlantic										
Branta canadensis interior					+	+	+		+	+
Mississippi										
Branta canadensis interior (Seney Refuge)					+	+	+			+
Branta canadensis interior (other localities)							+			+
Eastern Prairie†										
Anser caerulescens caerulescens	+		+		+	+	+	+		+
Branta canadensis hutchinsi	+	+					+			
Branta canadensis interior							+			+
Central										
Anser albifrons frontalis							+			+
Branta canadensis parvipes		+								
Pacific										
Olor columbianus										+
Anser albifrons frontalis				+						+
Anser caerulescens caerulescens							+			+
Anser rossi									+	+
Branta canadensis moffitti									+	+
Branta canadensis taverneri (?)										+
Branta canadensis minima						+	+			+

* The flyways are listed from east to west.
† Snow geese stopping to feed along the southwest coast of Hudson Bay in autumn are considered to belong to this flyway.

Probably too few birds were examined from the last two flyways to yield reliable figures.

The paucity of coccidian species in Canada geese of the Mississippi flyway is somewhat obscured by the occurrence of *E. fulva* and *E. hermani* in birds from the Seney Wildlife Refuge, Michigan. The Canada goose population at this refuge includes birds brought in from other flyways which live there permanently. Only *E. magnalabia* and *T. anseris* were found in 160 Canada geese from Horseshoe Lake, Illinois, and from the west shores of Hudson Bay and James Bay in Canada.

The lesser snow geese which feed along the southwest coast of Hudson Bay in autumn, and again more briefly in spring, carry five species of coccidia not found in the Horseshoe Lake flock of Canada geese, yet a portion of the latter also feed in the coastal areas for varying periods in late summer and autumn. The reasons for this lack of cross transmission between the two populations are not clear, particularly as three of the five species (*E. anseris*, *E. fulva*, and *E. hermani*) have already been recorded from *Branta*. However, the following factors may have some bearing on the problem: Most of the Canada geese which breed in the muskeg south of the coast do not frequent the coastal areas in spring, while in late summer and autumn only a portion of the flock reaches the coastal areas. The latter includes families of geese that have slowly descended the rivers, reaching the coast about the time the young are ready to fly. But, according to the Indians of the region, these families concentrate at the timber-line sector of the rivers instead of proceeding close to the coast where the type of marsh favored by the snow geese is found. Depending on the region in question, the timber line may be from several to 15 or more miles inland from the main feeding marshes of the lesser snow goose. Thus at least partial ecological separation of the two species would tend to hold cross transmission to a minimum.

Climatic conditions may also be a factor. Oocysts dropped by snow geese on the coastal marshes during the previous autumn would likely be killed by freezing in winter. Widespread flooding of the rivers due to ice jams at the time of the spring breakup would also cleanse areas around the lower reaches of the rivers.

Other unknown factors may also be operating. Doran's (10) experience with *Eimeria mohavensis* of the kangaroo rat, *Dipodomys panamintinus mohavensis*, in southern California is suggestive. He found this coccidium in 8.7% of 251 of these rats which he trapped and examined, and was able to transmit it experimentally to *D. merriami merriami* in 11 out of 12 trials. However, he never found it in 197 *D. m. merriami* which he trapped and examined, even though the two rat species are sympatric, nest near each other, have similar food habits, forage over the same range, have been taken in the same trap within half an hour, and *D. merriami* frequently robs food from the burrows of other species of kangaroo rats. Neither lack of susceptibility nor spatial or ecological separation, then, can account for the absence of *E. mohavensis* from *D. merriami*.

Another difference in parasitism between the *B. c. interior* populations of the South Atlantic and Mississippi flyways has to do with the gizzard worm, *Amidostomum anseris*. Herman and Wehr (22) reported that this nematode is extremely prevalent in Canada geese wintering on the coast of North Carolina, while Cowan and Herman (5) considered it to be an important cause of winter losses among them on Pea Island, North Carolina. It is uncommon, however, in the Canada geese at Horseshoe Lake, Illinois.

O'Roke (34) found *Amidostomum* sp. commonly in wild geese and ducks in California. This observation combined with those discussed above suggests a possible correlation between the occurrence of *Amidostomum* and *Eimeria truncata* in North America.

Because wild geese adhere strongly to the flyways in which they originate, which are for geese quite exact and of limited geographical scope, the dissemination of coccidia or other parasites from one flyway to another is not likely to be rapid. The data on incidence and prevalence of coccidia in wild geese bear this out, further reinforcing the validity of the flyway concept, at least as applied to geese.

Further studies are needed of larger numbers of geese in all North American flyways to map out more completely the distribution and prevalence of each coccidian species in the various host species, subspecies, and flyways. In addition, we can fully understand how well the distribution of the coccidia portrays anserine zoogeography and evolution only after such circumpolar species as *Anser caerulescens*, *A. albifrons*, and *Branta bernicla* have been studied both in North America and across Eurasia.

Further study of the Ross goose also appears desirable in order to ascertain whether there is a relationship between its low numbers and parasitism. The pathogenic *E. truncata* occurs in this goose, and O'Roke (34) found the gizzard worm, *Amidostomum*, in three of five Ross geese he examined in California. Coccidia are generally more pathogenic in young birds than in old ones, but observations of Ross goose broods on the breeding grounds at Perry River, Northwest Territories, in 1949 (by Hanson, Queneau, and Scott (18)) revealed no significant mortality during the first 2 weeks of life.

As a matter of fact, little attention has been paid even to the coccidia of the domestic goose. Most reports of coccidiosis in this bird have dealt with *E. truncata*, which brought itself into notice because of its pathogenicity.

It appears, however, that *Tyzzeria* is primarily a parasite of wild geese which seldom occurs in domestic geese. It is not unlikely that future cross-transmission experiments will show that the *Tyzzeria* of geese and ducks are the same; if so, the correct name would be *T. perniciosa* Allen, 1936 (1). *Eimeria magnalabia* and probably most of the other anserine species of *Eimeria*, even including *E. anseris* and *E. nocens*, are also probably primarily parasites of wild geese seldom occurring in domestic geese. Finally, it is tempting to speculate that *E. truncata* was originally a parasite of the graylag and domestic goose in Eurasia, and that it has reached North American wild geese relatively recently, entering from both the east and west.

There is little evidence as to whether coccidian transmission is greater on the breeding or the wintering grounds. Studies of the prevalence of coccidia when the birds arrive in the south and periodically thereafter are needed to determine the amount of transmission on the wintering grounds. Some information on the role of the breeding grounds in the transmission of coccidiosis is provided by our findings on *B. c. interior*. The 24 birds collected at Ft. Severn, Ontario, and Owl River, Manitoba, were all immature. Since they had never been south, it is clear that *Eimeria magnalabia* and *Tyzzeria anseris* (the two species of coccidia found in them) at least can be transmitted on the northern breeding grounds. In addition, our study on the wintering ground at Horseshoe Lake, Illinois, in November and December, 1954, and February, 1955, indicated no significant change in the infection rate during the interval.

Acknowledgments

We are indebted to many individuals and agencies for making this study possible. Foremost of these are Dr. C. H. D. Clarke, Mr. Campbell Currie, and Dr. Keith Reynolds of the Ontario Department of Lands and Forests, who provided the senior author with the opportunity of making studies of geese in northern Ontario in 1954. The results of these studies gave the incentive for widening the scope of the investigation. Mr. George Arthur of the Illinois Department of Conservation made collections from gosling Canada geese in northern Ontario and Manitoba. Mr. Timothy Nichol, then of the Manitoba Department of Mines and Resources, made collections in the York Factory area. Others who provided invaluable collections are Mr. Louis Lemieux of the Canadian Wildlife Service, Mr. Lewis G. Helms of the Missouri Conservation Department, and Mr. Vernon D. Stotts of Stephensville, Maryland. Dr. Ray C. Erickson, Mr. Gene H. Crawford, Mr. Vernon Ekedahl, Mr. Thomas C. Horn, and Mr. John C. Scharff of the United States Fish and Wildlife Service kindly provided fecal samples from geese wintering on federal wildlife refuges in California and southern Oregon.

Summary

In a survey of the coccidia of 362 wild geese and swans from Canada and the United States, *Eimeria anseris* was found in *Anser caerulescens caerulescens* and *Branta canadensis hutchinsi* (both new host records); *E. brantae* was found in *B. c. hutchinsi; E. fulva* was found in *A. c. caerulescens* (new host records); *E. hermani* was found in *A. c. caerulescens* (new host record) and *B. c. minima; E. magnalabia* (syn., *E. striata*) was found in *A. albifrons frontalis, A. c. caerulescens* (both new host records), *B. c. interior, B. c. hutchinsi*, and *B. c. minima; E. nocens* was in found in *A. c. caerulescens* (new host record); *E. truncata* was found in *Anser rossi* (new host record) and *B. c. moffitti; E. clarkei* n. sp. was found in *A. c. caerulescens; E. farri* n. sp. was found in *A. albifrons frontalis;* and *Tyzzeria anseris* was found in *Olor columbianus, A. a. frontalis, A. c. caerulescens, A. c. atlantica, A. rossi, B. bernicla hrota* (all four new host records), *B. c. interior, B. c. moffitti, B. c. taverneri* (?),

and *B. c. minima*. The sporulated oocysts of *E. anseris* and *E. nocens* are described for the first time. These species had previously been reported only in the domestic goose in Europe. Reports in the literature on the species of coccidia occurring in wild and domestic geese and their prevalence and geographic distribution are summarized with particular reference to North American flyways. *Tyzzeria anseris* is the most widely distributed coccidium, being found in all six flyways from which birds have been studied. *E. truncata* occurs on both the Atlantic and Pacific coasts, but has not been found in wild geese in the central part of the continent.

References

1. ALLEN, E. A. *Tyzzeria perniciosa* gen. et sp. nov., a coccidium from the small intestine of the Pekin duck, *Anas domesticus* L. Arch. Protistenk. **87**, 262–267 (1936).
2. ANONYMOUS. Twenty-seventh supplement to the American Ornithologists' Union check-list of North American birds. Auk, **69**, 308–312 (1952).
3. CHAKRAVARTY, M. and BASU, S. P. On a new coccidium, *Tyzzeria alleni* n. sp., from the intestine of the bird cotton-teal. Sci. and Culture (Calcutta), **12**, 106 (1946).
4. COOCH, G. Observations on the autumn migration of blue geese. Wilson Bull. **67**, 171–174 (1955).
5. COWAN, A. B. and HERMAN, C. M. Winter losses of Canada geese at Pea Island, North Carolina. Proc. Southeast. Assoc. Game Fisheries Comm. Meeting, 1955: 172–174 (1955).
6. CRITCHER, S. Renal coccidiosis in Pea Island Canada geese. Wildlife in N. Carolina, **14**(1), 14–15, 22 (1950).
7. DELACOUR, J. Preliminary note on the taxonomy of Canada geese, *Branta canadensis*. Am. Museum Novitates, No. 1537. 1951.
8. DELACOUR, J. The waterfowl of the world. Country Life, Ltd., London, Vol. 1. 1954.
9. DELACOUR, J. and MAYR, E. The family Anatidae. Wilson Bull. **57**, 3–55 (1945).
10. DORAN, D. J. Coccidiosis in the kangaroo rats of California. Univ. Calif. Publs. Zoöl. **59**, 31–60 (1953).
11. FARR, M. M. *Tyzzeria* sp. from wild geese and a wild duck. J. Parasitol. Suppl. **38**, 15 (1952).
12. FARR, M. M. Three new species of coccidia from the Canada goose, *Branta canadensis* (Linné, 1758). J. Wash. Acad. Sci. **43**, 336–340 (1953).
13. FARR, M. M. Renal coccidiosis of Canada geese. J. Parasitol. Suppl. **40**, 46 (1954).
14. FARR, M. M. and WEHR, E. E. Eimeria truncata associated with morbidity and death of domestic goslings. Cornell Vet. **42**, 185–187 (1952).
15. HANSON, H. C. A three year survey of *Ornithofilaria* sp. microfilariae in Canada geese. J. Parasitol. **42**, 543 (1956).
16. HANSON, H. C. and GRIFFITH, R. E. Notes on the South Atlantic Canada goose population. Bird-banding, **23**, 1–22 (1952).
17. HANSON, H. C., LEVINE, N. D., and KANTOR, S. Filariae in a wintering flock of Canada geese. J. Wildlife Management, **20**, 89–92 (1956).
18. HANSON, H. C., QUENEAU, P., and SCOTT, P. The geography, birds and mammals of the Perry River region. Arctic Inst. N. Amer. Spec. Publ. **3**, 1–96 (1956).
19. HANSON, H. C. and SMITH, R. H. Canada geese of the Mississippi flyway with special reference to an Illinois flock. Illinois Nat. Hist. Survey, Bull. **25**, 67–210 (1950).
20. HARDCASTLE, A. B. A check-list and host-index of the species of the protozoan genus *Eimeria*. Proc. Helminthol. Soc. Wash. D. C. **10**, 35–69 (1943).
21. HELLMAYR, C. E. and CONOVER, B. Catalogue of birds of the Americas and the adjacent islands: Part 1, No. 2 (Spheniscidae—Anatidae). Field Museum Nat. Hist. Zool. Ser. 13. Publ. 615. 1948.
22. HERMAN, C. M. and WEHR, E. E. Occurrence of *Amidostomum* in Canada geese. J. Parasitol. Suppl. **39**, 34 (1953).
23. KOTLÁN, A. Adatar a vízimadarac (kacsa, liba) coccidiosisának ismertéhez. Allatorvosi Lapok, **55**, 103–107 (1932) (*vide* Hardcastle (20)).
24. KOTLÁN, A. Zur Kenntnis der Kokzidiose des Wassergeflügels. Die Kokzidiose der Hausgans. Zentr. Bakteriol. Parasitenk. Abt. I. Orig. **129**, 11–21 (1933).
25. LERCHE. Nierencoccidiose bei Hausgänsen. Z. f. Infektionskrankh. parasit. Krankh. u. Hyg. Haustiere, **25**, 122–133 (1923).
26. LEVINE, N. D. *Tyzzeria* and *Eimeria* from the Canada goose. Proc. Am. Soc. Protozoöl. **2**, 17 (1951).

27. LEVINE, N. D. *Eimeria magnalabia* and *Tyzzeria* sp. (Protozoa: Eimeriidae) from the Canada goose. Cornell Vet. **42**, 247–252 (1952).
28. LEVINE, N. D. A review of the coccidia from the avian orders Galliformes, Anseriformes and Charadriiformes, with descriptions of three new species. Am. Midland Naturalist, **49**, 696–719 (1953).
29. LEVINE, N. D. and HANSON, H. C. The blood parasites of the Canada goose. J. Wildlife Management, **17**, 185–196 (1953).
30. LEVINE, N. D., MORRILL, C. C., and SCHMITTLE, S. C. Renal coccidiosis in an Illinois gosling. North Am. Veterinarian, **31**, 738–739 (1950).
31. LEWIS, H. F. Migrations of the American brant (*Branta bernicla hrota*). Auk, **54**, 73–95 (1937).
32. MANNING, T. H. Blue and lesser snow geese on Southampton and Baffin Islands. Auk, **59**, 158–175 (1942).
33. NIESCHULZ, O. Eine neue Kokzidienart bei der Hausgans. Zentr. Bakteriol. Parasitenk. Abt. I. Orig. **152**, 74 (1947).
34. O'ROKE, E. C. Intestinal parasites of wild ducks and geese. Calif. Fish Game, **14**, 286–296 (1928).
35. WEHR, E. E. and HERMAN, C. M. Age as a factor in acquisition of parasites by Canada geese. J. Wildlife Management, **18**, 239–247 (1954).

THE OCCURRENCE OF TRIMETHYLAMINE OXIDE IN FUNDULUS HETEROCLITUS[1]

JANE M. G. OGILVIE[2] AND A. A. WARREN

Abstract

The trimethylamine oxide present in trichloroacetic extracts of *Fundulus heteroclitus* muscle was determined indirectly by the colorimetric determination of trimethylamine as the picrate salt. The oxide content increases with the length of the fish and, while spawning, females showed larger contents than males. *Fundulus* kept in fresh or sea water and fed an oxide-free diet, showed an increase in oxide content which reached a high value in 35–45 days. Afterwards the oxide content declined and then rose again. The liver has small amounts present which tend to vary similarly. Injections of the oxide were recovered mainly in the excreta and to a lesser extent in the muscle and liver tissues. *Fundulus* starved for a month showed an increase in the oxide content of the muscle. It appears that trimethylamine oxide originates in muscle tissue as a result of two processes, one exogenous and one endogenous in origin.

Introduction

Trimethylamine and trimethylamine oxide have been isolated from a large variety of marine organisms. No explanation for the presence of this oxide in marine fish has been generally accepted. However, several theories on its origin, function, and metabolic fate have been proposed and were reviewed by Dyer (4).

For many years fresh-water organisms were considered to contain no trimethylamine oxide or merely traces of it. More recently Anderson and Fellers (1) reported trimethylamine oxide as well as trimethylamine to be present in small amounts in certain fresh-water species of fish. This report further complicated the study of trimethylamine oxide as previously most theories were based on the presence of this compound in the tissues of marine fishes and its absence from fresh-water forms.

The research reported in this paper is concerned with the trimethylamine oxide content of the muscle tissue of *Fundulus heteroclitus*, the killifish, to determine whether the oxide present in the tissue is exogenous or endogenous in origin.

Methods

Killifish were caught at Indian Point near St. Andrews, N.B., and brought to the laboratory in large cans of sea water. The supply of fish was kept in tanks of standing sea or tap water, which was changed at intervals. The temperature of the fish varied between 13° and 15° C. during the winter months. In captivity those fish not participating in special diet experiments were fed a trimethylamine oxide-free diet of crushed dog cubes (Gro-pup).

[1]Manuscript received May 10, 1957.
Contribution from the Department of Biology, University of New Brunswick, Fredericton, New Brunswick. The present paper is based on a thesis presented by Jane M. G. Ogilvie in partial fulfillment of the requirements for the degree of Master of Science of the University of New Brunswick. She was granted a Fisheries Research Board of Canada scholarship.
[2]Now Mrs. J. M. Manson. Present address: Department of National Health and Welfare, Ottawa, Ontario.

Determination of Trimethylamine and Trimethylamine Oxide

Muscle

The fish were killed by decapitation immediately before dissection so that no trimethylamine (TMA) could arise from reduction of the trimethylamine oxide (TMAO) by bacterial agents. Head, tail, and entrails were removed from the body. The muscle was then separated from the backbone and integument. When determinations were made on fish 5 cm. or less in length, only the head, tail, and entrails were removed. The remainder of the fish was cut into small pieces.

As all the fish were quite small, 3 to 9 cm. long, several fish of the same relative size had to be filleted in order to obtain a sufficiently large sample. Before each experiment, the fish were measured from head to tail and the mean length calculated.

Trichloroacetic acid extracts of fish muscle were prepared according to the extraction method of Dyer, Dyer, and Snow (5). A direct determination of TMA was made on a small part of the extract. The TMAO present in the remainder of the extract was reduced and the colorimetric determination of TMA as the picrate salt was followed (3). The TMAO content may be represented by the difference between the direct and indirect readings. The TMA content of muscle or liver was never more than 0.20 mg. TMA-N/100 g. of tissue and so it was always neglected in calculation of the oxide content.

Liver

Determinations on the liver were performed in essentially the same manner as those on the muscle. However, since the amount of liver worked up to an extract was usually less than 500 mg., Dyer's method was modified by reducing the absolute quantities of the materials.

Throughout the course of the experiments the TMAO content is expressed on a wet weight basis: mg. oxide-N/100 g. wet muscle or liver.

Observations and Results

Variation of the TMAO Content

The TMAO content of killifish muscle was determined at intervals during the summer months, each determination being made as soon as possible after the fish had been brought to the laboratory from St. Andrews. Males and females were differentiated for analysis. The results are shown graphically in Fig. 1, which depicts an increase in TMAO content of both sexes with an increase in length. The figure indicates that at the beginning of July (A) there is no difference in the oxide content of the sexes ($t=0.302$). Towards the end of July (B), however, the TMAO content of females is markedly higher than that of males of the same length ($t=2.400$*). This is the time of spawning. In the latter part of August (C) spawning is just about over and the oxide content of males is comparable to what it was

*The TMAO was prepared in the laboratory according to the method of Elliot (6).The resulting crystals were recrystallized to a constant melting point from absolute ethyl alcohol.

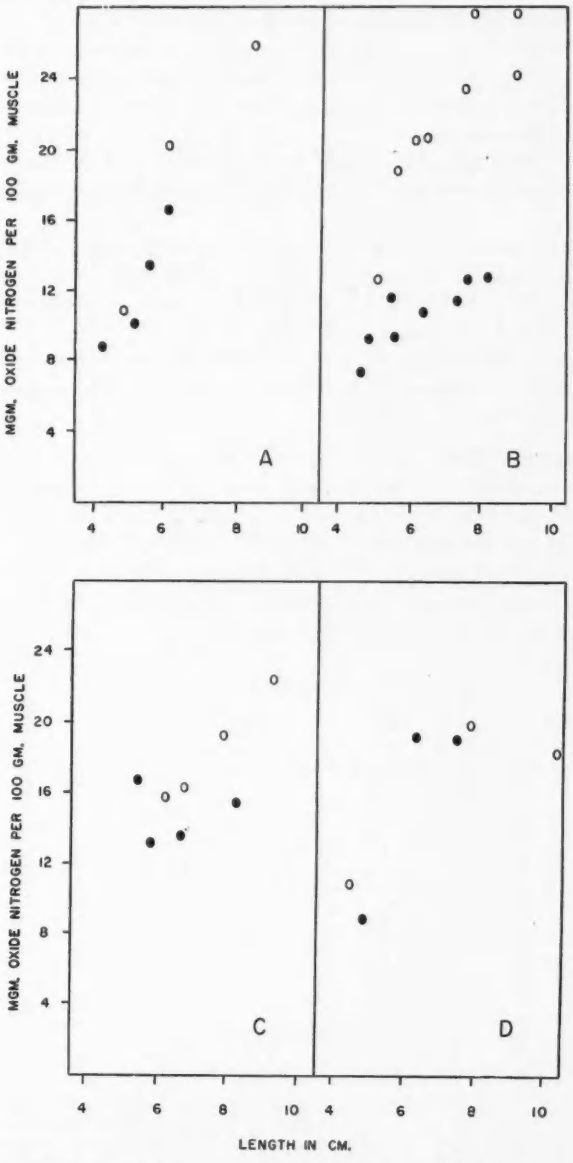


FIG. 1. The relationship between the TMAO content of killifish muscle and the mean length of the individuals. Males are indicated by closed circles and females by open circles.

A. Killifish caught on July 5, 1956.
B. Killifish caught on July 25, 1956.
C. Killifish caught on August 25, 1956.
D. Killifish caught on September 25, 1956.

previous to spawning. The resulting effect is a very slight and insignificant difference in the TMAO content of the muscles of males and females of the same length (t for the data in C = 2.286; t for the data in D = 1.726). A month later the external appearance of the fish indicated that the spawning season was definitely over.

Some very small fish were caught in September. These fish in which the TMAO was present in low concentrations were thought to have hatched this season.

A very few fish were caught in October. Four females, mean length 6.8 cm., contained a TMAO content of 21.24, while eight males, mean length 5.6 cm., showed a TMAO content of 20.40. As in September there is no difference in TMAO content attributable to sex.

Subsequently, fish caught on July 26, 1956, will be denoted as the "July" fish; those caught on August 25, 1956, as the "August" fish; and on September 25, 1956, as the "September" fish.

TMAO Content of Males and Females in Fresh and Salt Water

The fish discussed in this section were fed a TMAO-free diet. Some July fish that had been in sea water were placed in tap water. They adjusted themselves to this sudden change in salinity with no difficulty. At intervals of approximately one month the TMAO content of the muscle was determined. The results are presented graphically in Fig. 2, which shows a decrease in TMAO content of the muscle after the fish have been in fresh water for

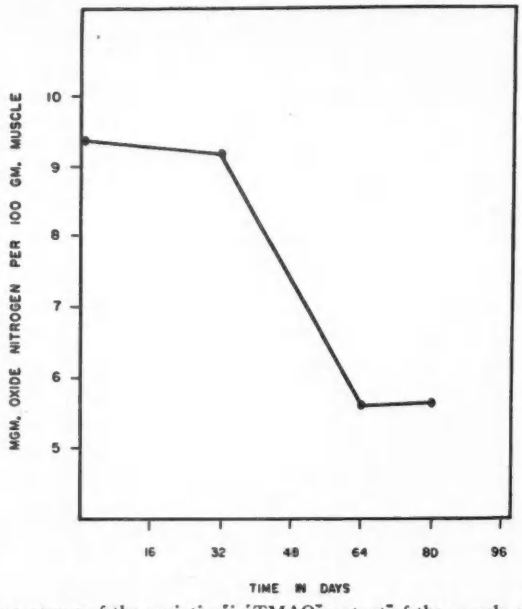


FIG. 2. Time course of the variation in TMAO content of the muscle of "July" male killifish kept in fresh water.

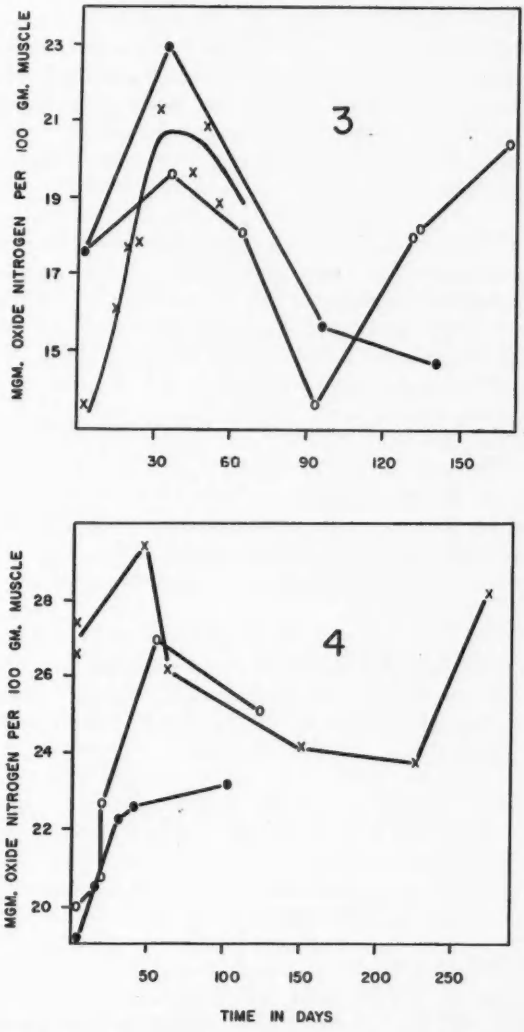


FIG. 3. Time course of the variation in TMAO content of the muscle of "August" killifish.
Females in sea water are indicated by closed circles.
Females in fresh water are indicated by open circles.
Males in sea water are indicated by crosses.

FIG. 4. Time course of the variation in the TMAO content of the muscle of killifish in sea water.
"September" females are indicated by open circles.
"September" males are indicated by closed circles.
Fall killifish are indicated by crosses.

35–64 days. There is a 40% drop in the oxide content. Twenty days later the content still showed the same low value. An insufficient number of fish prevented a complete repetition of this experiment with fish in sea water. However, two determinations were made with females. Initially 14 females, mean length 5.6 cm., contained a TMAO content of 18.70. Following a 73-day period in sea water eight females, mean length 5.7 cm., showed a TMAO content of 10.06. This is a 46% drop in the oxide content of the muscle.

August female killifish, in both fresh and sea water show an initial increase in TMAO content occurring after about thirty days, followed by a drop (Fig. 3). The experiment with the fish in sea water was carried on for 141 days. At the end of this period the oxide content was still low. However, the corresponding experiment in fresh water continued for a longer duration. Here, the drop in oxide content is followed by an increase, so that after 134 days the content has risen to a value approximately equal to the initial value. It was during this period that the fish were not eating well. At feeding time they did not come to the surface of the water and look for food but remained barely moving at the bottom of the tank. TMAO determinations were also performed on August males in sea water. The determinations were made more frequently and over a shorter period. The results are represented in Fig. 3, the curve being fitted by eye. A gradual increase in oxide content is again noted.

In a preliminary experiment a similar variation in TMAO content of the muscle was noted. The killifish used for the experiment were kept in sea water and had been brought to the laboratory on two occasions. Oxide determinations showed that these two groups of fish had similar TMAO contents and, consequently, were considered to be one and the same group in this respect. No distinction was made between males and females as these fish had been caught late in the season. Fig. 4 shows this variation graphically. The increase in TMAO during the first 35 days was also observed in September males and females, kept in sea water (Fig. 4). Experiments with the females were performed long enough to demonstrate the decrease subsequent to the initial rise in TMAO content.

TMAO Determination of the Liver

Determinations of TMAO were also made on the liver of fish fed a TMAO-free diet and kept in sea water. The results obtained demonstrate that the oxide is present in very small amounts but tends to vary in the same manner as that of the muscle (Table I).

Effect of Diet on TMAO Muscle Content

In the above experiments the killifish have been on a diet of crushed dog cubes. Now some were fed a diet containing no protein, others were put on normal and high protein diets, while still others were denied food. The fish were fed daily 500 mg. of the prescribed diet, which was sprinkled over the surface of the sea water. The diets were prepared according to the

formulae in the Diets Manual of Nutritional Biochemicals Company. The experiment ran for a month. The high protein diet had a severe effect on the appearance and metabolism of the fish. These fish developed a reddish color around their gills on the third day, and the greater number had died by the seventh day. The experiment was repeated and on the seventh day the fish were tested for the TMAO content of the muscle. No change was found.

TABLE I

TMAO CONTENT OF LIVER

Fish	Sex	No. of fish extracted	Mean length, cm.	Days in sea water	TMAO content of liver mg. oxide-N/100 g.
August	Female	5	7.9	2	4.21
	Male	3	8.3	3	1.74
	Male	6	7.1	16	2.77
	Male	12	6.5	25	2.38
September	Female	1	10.5	1	1.19
	Female	4	7.1	27	1.26
	Female	4	7.0	57	1.78
	Female	2	8.1	62	2.33
	Male	4	7.4	7	1.19
	Male	4	6.8	45	2.09

TABLE II

EFFECT OF DIET ON TMAO MUSCLE CONTENT

Sex	No. of fish extracted	Mean length, cm.	Diet	Initial TMAO mg. oxide-N/100 g.	Final TMAO mg. oxide-N/100 g.
Male	8	5.8	Gro-pup	20.40	
Female	4	6.8	Gro-pup	21.29	
Male	5	6.7	Non-protein		23.58
Male	5	6.1	Non-protein		22.65
Male	3	7.7	Normal protein		22.53
Female	2	7.0	Normal protein		21.74
Male	1	6.4	Fasted		26.52
Female	5	7.0	Fasted		29.07

The results of the other diets are drawn up in Table II. Feeding killifish these diets for 1 month had no significant effect on the TMAO content of the muscle. However, those fish that had been starved showed a marked increase in TMAO content.

These results led to an experiment consisting of starving some August females after they had been in fresh water for 92 days. The TMAO content of seven females, mean length 7.2 cm., was 13.59 before starvation was begun. After 1 month of starvation the TMAO content had risen to 26.39 for two females (mean length 7.3 cm.) as compared to 18.02 for three females (mean length 6.9 cm.) undisturbed by this starvation.

TABLE III

INJECTION OF TMAO*

Initial / Final mg. oxide-N/ 100 g. muscle	Initial / Final mg. oxide-N/ 100 g. liver	TMAO injected, mg.	Muscle weight, g.	Liver weight, mg.	Muscle recovery of TMAO, mg.	Liver recovery of TMAO, mg.	Water recovery of TMAO, mg.	Total % recovery of TMAO
26.97 (2) / 36.47 (2)	2.33 (2) / 10.40 (2)	2.0	3.0	330	0.29	0.02	1.66	98.5
22.62 (4) / 29.97 (3)	2.33 (4) / 21.13 (3)	3.0	3.1	320	0.23	0.06	1.40	56.3
17.74 (15) / 22.11 (12)	Not tested	6.0	8.3	484	0.36		4.47	82.5
23.20 (4) / 35.60 (2)	5.88 (4) / 35.82 (2)	2.0	1.9	238	0.23	0.07	1.55	92.5

* The numbers in parentheses indicate the number of fish used in the corresponding determination.

Injection and Feeding Experiments

Betaine, choline, and methionine were injected into the body cavity of fish to determine whether these chemicals were precursors in the synthesis of TMAO; TMAO* was injected into other fish to follow its fate. After being placed in a small volume of sea water for 24 hours the fish were killed and the muscle, liver, and water were tested for TMAO. Any TMAO found in the water could be attributed to TMAO excreted by the fish. No increase in TMAO either in the fish or the water was detected following the injections of betaine, choline, and methionine. There was an increase in the TMAO content of both muscle and liver following the injection of TMAO, but the greatest recovery of the oxide was found in the water into which it had been excreted (Table III).

Some fish that had previously been on a TMAO-free diet were placed on a diet containing TMAO, an homogenate made from sole fillets. The homogenate was fed to seven fish in sea water over a period of 12 days. The feeding was performed with the aid of a small dropper, which had been calibrated and was inserted into the fish's mouth. A small recovery of the oxide was found in the muscle. Some other fish were fed a solution of TMAO in the same manner. This experiment ran for 14 days. Again, a small recovery was noted in the muscle (Table IV).

TABLE IV

FEEDING EXPERIMENTS

Initial number of fish extracted	Initial TMAO mg. oxide-N/100 g. muscle	Diet	Total TMAO fed mg. oxide-N/100 g. muscle	Final number of fish extracted	Final TMAO mg. oxide-N/100 g. muscle	% recovery of TMAO in muscle
6	29.37	Sole homogenate	64.3	7	31.07	2.5
9	26.17	TMAO solution	462.2	8	36.08	2.2

Examination of the Stomach Contents

Food organisms in the two loops of the intestine were examined microscopically. Amphipods belonging to the family Orchestiidae were found most abundantly. A few Hemiptera belonging to the family Corixidae (water boatman) were present along with a few polychaete worms and some small molluscs. Killifish favor amphipods since most of the stomachs examined contained only amphipods. A good sized fish, 7.0–7.5 cm. long, may contain two to three dozen. Small fish 4 cm. long may contain 10 amphipods in the two loops of the intestine.

Discussion

The results have shown that when killifish fast the oxide content of their muscle tissue rises. While the fish are on a TMAO-free diet the oxide content first rises, then falls, after which it rises again to its normal level. Apparently TMAO normally originates in marine fish muscle tissue as the result of two

processes, one exogenous and one endogenous in origin. When the exogenous supply of TMAO is cut off by feeding the fish a TMAO-free diet, the endogenous process carries on alone and we find a rise in TMAO content for about 30–45 days. Then this endogenous process presumably is overtaken by the failure of the exogenous source of TMAO and the TMAO content falls. Since a certain amount of TMAO must be necessary for normal functioning of the metabolism of marine fish, the same endogenous process or possibly a second one, begins to build up TMAO again. This would correspond to the rise to the normal level that has been observed. The rise in TMAO in the fasting fish probably corresponds to the endogenous rise noticed during the first 30–45 days on a TMAO-free diet.

Experimental evidence has shown that the TMAO content of killifish muscle is subject to variation before, during, and immediately following the spawning period. The metabolic function of this oxide may be in some way related to the variation of the oxide content with sex and length while spawning. It is possible that male and female possess different rates of feeding, which would have to be considerable to cause a difference in oxide content as great as has been demonstrated. The two intestinal loops of 100 killifish caught in July were examined. There appeared to be no difference in the amount of feeding between the sexes. Although this examination was only qualitative, it appears that both males and females fed normally. Newman (7) says that during spawning the females have to sacrifice much of their vitality to egg production and become rather sluggish. It appears that fish, in which metabolism is low, produce more TMAO.

Killifish winter in a more or less sluggish state on the bottoms of deeper holes or creeks (2). Fish kept in laboratory tanks took less food during December and January than normally and were in a sluggish state. At this time there was an increase in the oxide content. Perhaps seasonal changes in metabolism of the fish are being reflected in TMAO production.

Ronald and Jakobsen (10) reported the presence of TMAO in milt and roe of herring. The amount of oxide in the milt was about three times as great as that in roe. It may be that the oxide is transferred from the muscle to the milt. However, no experiments have been done correlating the oxide content of muscle and milt over the year. Poller and Linneweh (9) also found TMAO in the roe of herring. Two determinations for amine and oxide were made on the roe of killifish; amine contents of 0.28 and 0.82 mg. amine-N/100 g. roe and corresponding oxide contents of 1.40 and 0.60 mg. oxide-N/100 g. roe were found. The determination resulting in a higher amine and lower oxide content was made on roe that had been in the refrigerator for a day.

Eggs of killifish fertilized around the last of July would hatch in 9–18 days, the incubation period depending upon the temperature. On hatching the larva is 7.0 to 7.7 mm. long. A fry of about 20 mm. resembles its parents. By examination of the stomach contents it has been found that these killifish feed almost exclusively on amphipods, which contain 3.06 mg. oxide-N/100 g. (8). At the end of September these fish were 4.0 to 4.5 cm. in length and had oxide values ranging from 7.24 to 10.85 mg. oxide-N/100 g. This is

approximately 50 days after the fish have been hatched. Assuming that the fish eat 12 amphipods of average size and weight every day from the time they are hatched and that 85% of the oxide is excreted and 15% of the oxide is stored in the muscle every day, it would take 200 days for the TMAO content to build up to 10 mg. oxide-N/100 g. No absolute decision can be reached on these assumptions since they are only theoretical. However, since it takes only 50 days for young killifish to accumulate this amount of TMAO, a process giving rise endogenously to TMAO must be in operation in addition to the exogenous source of the oxide.

Acknowledgments

We wish to express our thanks to Dr. W. J. Dyer and his laboratory assistant, Miss Doris Fraser of the Fisheries Research Board of Canada Station, Halifax, N.S. for instruction in Dyer's method of trimethylamine oxide determination. We also acknowledge the assistance of the personnel of the Biological Station of the Fisheries Research Board of Canada at St. Andrews, N.B., who supplied sea water and occasionally helped in catching the killifish.

References

1. Anderson, D. W. and Fellers, C. R. Occurrence of trimethylamine and trimethylamine oxide in fresh water fishes. Food Research, **17**, 472–474 (1952).
2. Bigelow, H. B. and Schroeder, W. C. Fishes of the Gulf of Maine. Fishery Bull. of the Fish and Wildlife Serv. **53**, 162–164 (1953).
3. Dyer, W. J. Colorimetric determination of trimethylamine as the picrate salt. J. Fisheries Research Board Can. **6**, 351–358 (1945).
4. Dyer, W. J. Trimethylamine oxide content of fish and marine invertebrates. J. Fisheries Research Board Can. **8**, 314–324 (1952).
5. Dyer, W. J., Dyer, F. E., and Snow, J. M. Trimethylamine oxide estimation. J. Fisheries Research Board Can. **8**, 309–313 (1952).
6. Elliot, P. R. Reduction of trimethylamine oxide in dogfish flesh. Food Research, **17**, 225–234 (1952).
7. Newman, H. H. Spawning behaviour and sexual dimorphism in *Fundulus heteroclitus* and allied fish. Biol. Bull. **12**, 314–348 (1907).
8. Norris, E. R. and Benoit, G. T. Occurrence of trimethylamine oxide in marine organisms. J. Biol. Chem. **158**, 433–439 (1945).
9. Poller, K. and Linneweh, W. Über das vorkommen von trimethylamin-oxyd in *Clupea harengus*. Ber. Chem. Ges. **59**, 1362–1365 (1926).
10. Ronald, O. A. and Jakobsen, F. The trimethylamine oxide content of Norwegian marine products and the fate of trimethylamine oxide during bacterial decomposition of raw materials for canning. J. Soc. Chem. Ind. **66**, 160–166 (1947).

THE METAZOAN PARASITES OF THE HETEROSOMATA OF THE GULF OF ST. LAWRENCE

II. ENTOBDELLA CURVUNCA SP. NOV. (TREMATODA: CAPSALIDAE)[1]

KEITH RONALD

Abstract

Entobdella curvunca sp. nov. (Trematoda: Capsalidae) is described from *Hippoglossus hippoglossus* from the Gulf of the St. Lawrence.

The common halibut (*Hippoglossus hippoglossus*) of the northwest Atlantic Ocean has been recorded previously as carrying only one representative of the genus *Entobdella* (Blainville, 1818), namely *Entobdella hippoglossi* (Müller, 1776). This parasite has been reported from almost every area in which the host has been taken. The record of the species described below is, therefore, interestin gin that no mention has been made of it in the literature, suggesting that this parasite is limited to the Gulf of St. Lawrence from where few reports have been made on the monogenetic trematode fauna of fish hosts.

In the present survey specimens of *E. hippoglossi* were often found on the skin of *Hippoglossus hippoglossus*, as were smaller ectoparasitic trematodes. A study of the known species of *Entobdella* indicated that although the writer's specimens belong to the genus by virtue of the presence of glandular areas on the cephalic lobe, they do not actually correspond to any of the published descriptions and are, therefore, described as a new species.

Entobdella ***curvunca*** sp. nov.

The color of the living trematode is almost pure white, with few pigmented areas. Body the shape of an elongate ellipse, 7 to 13 mm. long by 3 to 6 mm. wide; cephalic lobe definitely emarginated from the rest of the body by lateral constrictions. Anterior haptors poorly formed into elliptical, slightly depressed glandular areas; line of demarcation weakly defined between the two prohaptors. Opisthaptor with the characteristics of the genus, sucker-like, 1.5 to 2.5 mm. in diameter, surrounded by marginal membrane 0.055 to 0.075 mm. wide. Ventral surface concave, posterior two-thirds bearing radiating rows of prominent papillae which decrease in size as they approach the center of the opisthaptor. Opisthaptor armed with three pairs of large hooks (anchors) and 14 marginal hooklets. Hooks of the first pair pointed, flat-bladed, 0.390 to 0.455 mm. in length and 0.091 to 0.143 mm. wide at the broadest point. Second pair of hooks longer: 0.54 to 0.99 mm., tips of which are recurved and pointed; roots twisted and slender, 0.052 to 0.091 mm. wide. Third pair of hooks recurved into fine points posteriorly with curved indentations anteriorly, 0.085 to 0.119 mm. long, 0.025 to 0.040 mm. wide. Marginal hooklets twisted, approximately 0.055 mm. long by 0.004 mm. wide.

[1] Manuscript received August 13, 1957.
Contribution from Département des Pêcheries, Quebec No. 58, Station de Biologie Marine, Grande-Rivière, Qué., and the Institute of Parasitology, McGill University, Macdonald College, Macdonald College P.O., Que., Canada.

Mouth ventral, slightly posterior to marginal constrictions limiting prohaptor from the body. Pharynx 0.40 to 0.80 mm. long by 0.46 to 0.58 mm. wide. Intestine bifurcates into two main arms close behind the pharynx, the two arms passing the length of the body, with four main branches anteriorly and ten branches laterally. Junction anterior to the opisthaptor, between main cañals.

Common genital atrium at left and slightly posterior to lateral marginal constrictions of prohaptor. Cirrus pouch present, elongated and rounded, lying between pharynx and ovary, with its base closer to anterior extremity of ovary than to posterior edge of pharynx. Ovary broadly elliptical, 0.60 to 0.85 mm. long by 0.56 to 0.76 mm. wide.

Both testes hexagonal in shape, touching on the median line, equatorial in position, 1.08 to 1.32 mm. long by 0.72 to 1.06 mm. wide. Vitellaria well developed, follicular, occupying almost entire body area, less closely

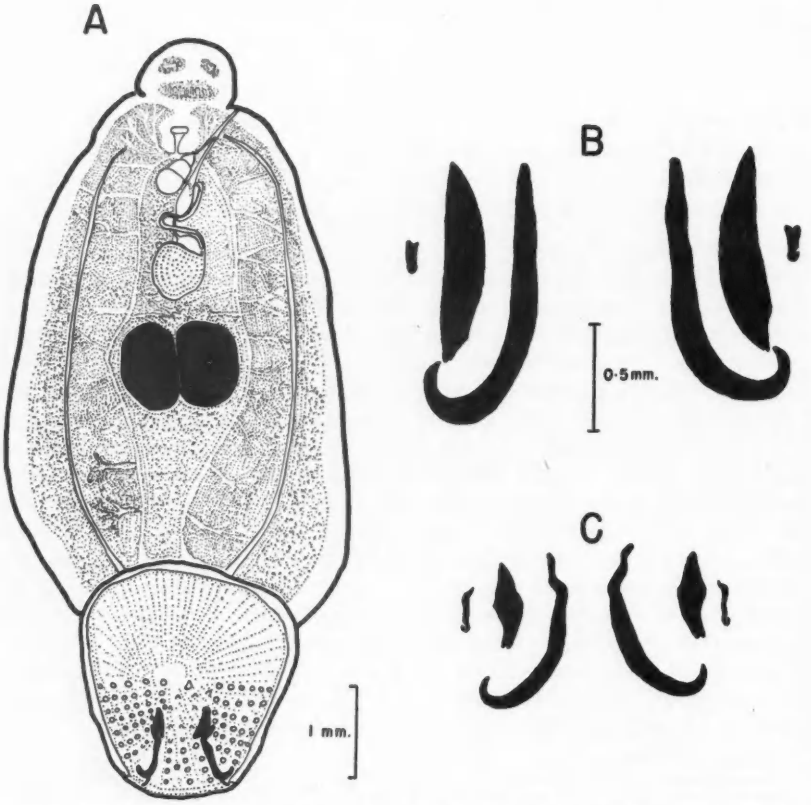


FIG. 1. (A) *Entobdella curvunca*. (B) The three pairs of hooks of the opisthaptor of *E. hippoglossi*. (C) The three pairs of hooks of the opisthaptor of *E. curvunca*.

TABLE I

COMPARATIVE SIZE AND SHAPE OF FOUR SPECIES OF *Entobdella*

Species	Body			Opisthaptor	Hooks					
					First pair		Second pair		Third pair	
	Shape	Length, mm.	Width, mm.	Diameter, mm.	Shape	Length, mm.	Shape	Length, mm.	Shape	Length, mm.
E. bumpusii	Ovoid	8.5–12.5	5.0–8.3	2.4–3.0	Robust, slightly curved, gradually attenuated anteriorly	0.646–0.795	Slender, tips recurved	0.731–0.765	Tips slender and recurved	0.076
E. curvunca	Elongated ellipse	7.0–13.0	3.0–6.0	1.5–2.5	Pointed anteriorly and flat-bladed	0.390–0.455	Tips recurved and pointed	0.540–0.990	Recurved to fine points posteriorly, curved indentations anteriorly	0.085–0.119
E. hippoglossi	Elliptical	13.0–24.0	3.6–11.0	3.6–4.8	Spear-head shaped, median margin, slightly curved	0.510–0.610	Slender, tips recurved	0.815–0.935	Tips slender, recurved	0.095–0.122
E. squamula	Elliptical	3.6–18.0	3.0–10.0	1.5–2.9 by 1.4–2.9	Spear-head shaped	0.197–0.390	Tips recurved	0.595–0.986	Tips slender, recurved	0.121–0.163

packed laterally, limited anteriorly by anterior margin of pharynx, posteriorly by opisthaptor. Uterus slightly coiled containing few eggs. Vagina opens behind the common genital aperture.

Host: *Hippoglossus hippoglossus* (Linné, 1758).
Location: Skin.
Locality: La Tabatière, north shore of the Gulf of St. Lawrence; Anticosti Island; Brion Island, Magdalen Islands.
Incidence: Fourteen of a total of 57 halibut examined carried *E. curvunca*.

The three species of *Entobdella* described previously from the northeastern Atlantic have been redescribed by Price (2). The descriptions and redescriptions are used here to show the morphological variations between *E. curvunca* and *E. hippoglossi*, *E. squamula*, and *E. bumpusii* (Table I). The size of the hooks cannot be used, in the author's opinion, as a diagnostic criterion owing to the overlap of the measurements found in dissimilar species; for example, *E. hippoglossi's* third pair of hooks measure 0.095 to 0.122 mm. in length, those of *E. curvunca* 0.085 to 0.119 mm., but the shape differs as shown in Figs. 1B and 1C, which were drawn from specimens of the two species taken from the same host.

Body size and shape are of little use in specific determination. In Table I it will be seen that the measurements of any one species would fit into the prescribed limits of the other three. The diameter of the opisthaptor is a little more critical in that the smallest measurement for *E. hippoglossi* is larger than the largest diametric measurement of the other three; the latter, however, do overlap. The ratios of the mean length of the opisthaptor to the mean body length are inconclusive; the only major distinction is shown by *E. hippoglossi* with a 50% variation from the mean, the other three all falling within a 20% distribution.

The only persistent specific characteristic is to be found in the shape of the hooks. Caution should also be used here as Brinkman (1) has indicated that allometric growth may supervene early in the development of the trematode *E. squamula*. The shape of the second and third pairs of opisthaptorial hooks is the primary criterion used to distinguish *E. curvunca* (Fig. 1C) from the other members of the genus. The curved roots of these appendages differ widely from those found in the other species, but the trematode, by virtue of its morphological similarities, cannot be raised above specific level.

References

1. Brinkman, A. Some Chilean monogenetic trematodes. Reports of the Lund University Chile Expedition 1948–1949, (6). Lunds Univ. Årsskr. n.F. Avdel. 2. **47** (11), (1952).
2. Price, E. W. North American monogenetic trematodes. III. The family Capsalidae (Capsaloidea). J. Wash. Acad. Sci. **29**, 63–92 (1939).

STUDIES ON THE ANATOMY OF LYMNAEA HUMILIS SAY[1]

BRUCE M. MCCRAW[2]

Abstract

The topographic anatomy of *Lymnaea humilis* is described, giving the relative positions of the various internal organs. A description of the anatomy of the cephalic hemocoele is also given. Like *Lymnaea stagnalis* the central nervous system of *L. humilis* consists of the paired cerebral, buccal, pedal, pleural, and parietal ganglia and the unpaired abdominal ganglion. The nerves arising from the central nervous system are described and particular attention was devoted to the nerves arising from the pedal ganglia and innervating the foot; fourteen new nerves are named or described. The reproductive system of *L. humilis* is divided into: (*a*) the ovotestis and its duct, (*b*) the female system, and (*c*) the male system. In reproductively active snails, the female system is the largest of the three portions, and consists of the uterus, oothecal gland, vagina, and seminal receptacle. An accessory structure, the albumen gland, is very large in *L. humilis*. The male system consists of the upper and lower prostate, vas deferens, and male copulatory organ. The connections between the hermaphrodite duct and the male and female systems show considerable structural consolidation compared to these connections in *L. stagnalis*. The muciparous gland is not a distinct entity in *L. humilis*.

Introduction

In recent years, the anatomy of gastropods has received increased attention because certain of the internal structures are important in understanding the systematics of this group of molluscs. In his critical study of the Lymnaeidae, Hubendick (18) based much of his revision of this family on the size and shape of parts of the reproductive system. However, there is still evident need for comparative anatomical studies upon many of the Lymnaeidae, as well as other Pulmonata. Moreover, a knowledge of snail morphology is essential in studying trematode life cycles, as it forms an important basis in the interpretation of the developmental stages of flukes.

An excellent bibliography on snail anatomy is contained in the paper by Carriker (12) and only the more pertinent works will be mentioned here. The topographic anatomy of *Lymnaea stagnalis appressa* has been described by Carriker (12), who made an exhaustive study on the structure of the alimentary canal of this snail. The work of Carriker and Bilstad (13) on the histology of the digestive system of *L. stagnalis* is an extensive one.

The nervous system of the Lymnaeidae has been studied by Lacaze Duthiers (21), Baker (5, 6), Bargmann (10), Elo (15), and most recently Carriker (12). One of the most thorough investigations into the nervous system of any pulmonate is that done by Schmalz (22) on *Helix pomatia*. Lacaze Duthiers' paper (21) was the first extensive work dealing with the nervous system of the Lymnaeidae alone. He described the principal ganglia and nerve trunks of *Lymnaea pereger* and *L. stagnalis*. Elo (15) dealt particularly with the

[1]Manuscript received July 10, 1957.
Contribution from the Division of Biology, Ontario Veterinary College, Guelph, Ontario, and from the Museum of Zoology, University of Michigan, Ann Arbor, Michigan. This study is a portion of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the University of Michigan.
[2]Present address: Ontario Veterinary College.

ramifications of the main nerve trunks, especially those serving the penis, mantle, and intestine of *L. stagnalis*. He also attempted to homologize some of the principal nerves of *L. stagnalis* and *H. pomatia*. Although Carriker (12) was concerned primarily with the innervation of the alimentary canal of *L. stagnalis*, he also studied most of the remaining principal nerves. In general, the findings of these two authors agree reasonably well. However, Carriker (12) clarified the nomenclature of the central nervous system of *L. stagnalis*, basing as much as possible on the names used by former investigators. Carriker's (12) system of nomenclature is used in the present study, except where new nerves are described.

The gross morphology of the reproductive tract of *Lymnaea* has been studied by Baudelot (11) and Crabb (14), who also briefly mentioned the histology as did Klotz (20). The first accurate description of the reproductive system of *L. stagnalis* was given by Crabb (14). More recently, Holm (17), in his detailed histological studies, elaborated and clarified many of the functional aspects. The ovotestis (hermaphrodite gland) of *L. stagnalis* has been investigated by Archie (4) and the embryology of the reproductive system as a whole by Fraser (16).

The reproductive system of members of the Planorbidae has been investigated by Baker (8), Hubendick (19), and also by Abdel-Malek (2, 3), who gave a brief but thorough coverage of the literature on these structures in other groups of Gastropoda.

Some aspects of lymnaeid systematics, and, in particular, the position of *Lymnaea humilis* Say deserve special comment. Recently, Hubendick (18) questioned the extensive use of some morphological features in the taxonomy of the family Lymnaeidae. One of these is the shell, which shows great intraspecific variation. This variation is well exemplified in his biometric analysis of the shell *Lymnaea peregra*. Shell size and thickness, as well as zebrine coloration and the position of growth lines, do not possess taxonomic value because they are less dependent upon hereditary conditions than upon environmental factors. Another is the radula, the use of which as a key character is limited by the fact that divergences in its structure are generally so small that reliable observations are difficult to make. This is further complicated by the smallness of the radula itself. Previously, considerable importance has been attached to variations in the shell and radula (6, 7). However, the findings of Hubendick (18) indicate that extreme care is necessary in using these characters in the systematics of the Lymnaeidae. As a result of critical study, the following species recognized by Baker (6) have been linked by Hubendick (18) to *Lymnaea humilis* Say: *doddsi* Baker, *umbilicata* Adams, *cyclostoma* Walker, *parva* Lea, *parva sterki* Baker, *owascoensis* Baker, *pilsbryi* Hemphill, *ferruginea* Haldeman, *humilis modicella* Say, *humilis rustica* Lea, *obrussa* Say, *obrussa peninsulae* Walker, *obrussa exigua* Lea, *obrussa decampi* Streng, *galbana* Say, and *petoskeyensis* Walker. Hubendick (18) was hesitant to link *caperata* Say with *Lymnaea humilis*. *L. caperata* is recognized as a pond or woodland pool inhabitant.

Material for the present study was collected in the region of Ann Arbor, Michigan, principally from two localities. Both were on the Raisin River. One was located 3 miles southeast of Clinton, at Newburg, in Lenawee County; the other 1 mile northwest of Clinton, in Washtenaw County.

Materials and Methods

For both gross anatomy and histological studies it was necessary to relax snails prior to preservation or fixation. Menthol crystals were found to be satisfactory for relaxation (1). Living snails were placed in a stender dish and powdered menthol crystals were spread over the surface of the water. Best results were obtained by then refrigerating the menthol-treated snails for 18 to 24 hours. At room temperature sufficient relaxation occurred in 2 to 4 hours. However, at room temperature some menthol-treated snails occasionally died and the resultant degenerative changes made tissues unsatisfactory for sectioning. As soon as the snails failed to contract when placed in preservative or fixative they were judged to be sufficiently relaxed.

A 10% aqueous solution of formaldehyde was the fixative used most frequently. Gilsons and Bouins were also used. The relaxed snails were placed directly into the fixative and transferred after 24 hours to 70% alcohol. The alcohol was changed again after 24 hours to obtain a more complete removal of formaldehyde.

A Petri dish, partially filled with wax, served as a dissection tray. 'Minuten-nadeln', jeweller's forceps, and standard laboratory equipment were used as dissecting instruments. Tiny scalpels were made from the 'minuten-nadeln' by grinding them down on a fine grade of stone. Both the needles and the scalpels were inserted into balsawood handles. Equal parts of glycerin and 70% alcohol were used as a dissecting fluid for relaxed and preserved snails. This solution kept the tissues soft and easy to work. Relaxed living snails were also dissected. A few of these snails were raised in the laboratory; however, most were field specimens. These field snails were remarkably free of parasites.

Serial sections of both the entire animal and individual organs were made to confirm the identification of dissected structures. Acid alcohol was used for the removal of the shell, and gas trapped under the mantle cavity was removed by vacuum pump. If the gas were not removed, inadequate infiltration of wax resulted. Sections 8 or 10 to 50 μ were made and these were stained with Harris's hematoxylin and orange G and mounted in Canada balsam.

Topographic Anatomy

The description of the topographic anatomy of *L. humilis* (Figs. 1 to 6) is based upon that of a relaxed snail with the following shell dimensions: length 10.2 mm.; greatest width 5.5 mm.; length of aperture 5.7 mm.; width of aperture 5.1 mm. *L. humilis* of these dimensions has about five whorls and may be described as an 'average' size for a snail which has overwintered in the region of Ann Arbor, Michigan, and is 8 or 9 months old.

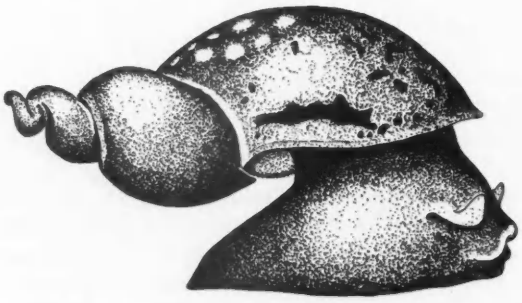
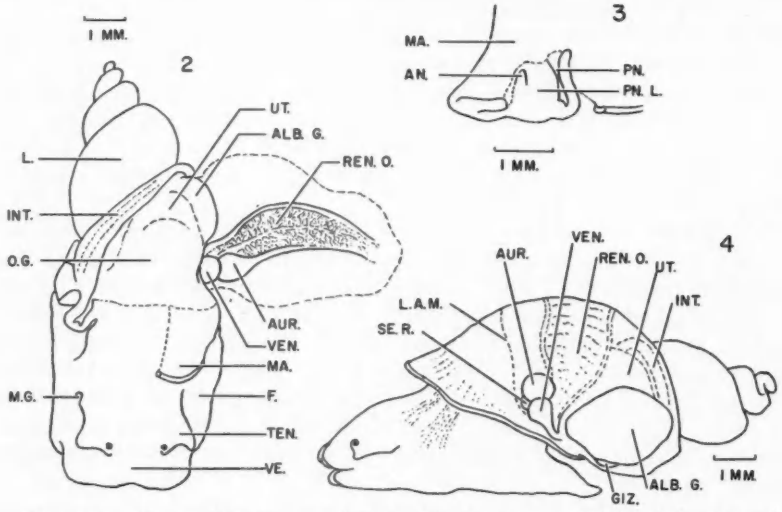


FIG. 1. *L. humilis* with the shell removed to show mantle pigmentation. Note the conspicuous bar of pigment parallel to the edge of the mantle.



FIGS. 2–4. Topographic features of *L. humilis* after removal of the shell. FIG. 2. Dorsal view with mantle cut. FIG. 3. Mantle partly cut to show anal and mantle cavity openings. FIG. 4. Lateral view of *L. humilis*.

A.—first portion of the hermaphrodite gland duct
AB.G.—abdominal ganglion
ALB.G.—albumen gland
ALB.G.D.—albumen gland duct
AN.—anus
AO.N.—aortic nerve
A.P.N.—anterior pedal nerve
A.PL.N.—anterior pallial nerve
AUR.—auricle
A.V.N.—anterior velar nerve
B.—second portion of the hermaphrodite gland duct
BC.C.—buccocerebral connective
B.G.—buccal ganglion
B.M.—buccal mass
B.R.M.—buccal retractor muscle
C.—third portion of the hermaphrodite gland duct
C.L.—commissural lobule
CO.N.—columellar nerve
C.P.N.—central pedal nerve
DB.N.—dorsobuccal nerve
DL.N.—dorsolabial nerve
D.M.—dorsal mandible
E.—eye
E.P.N.—external pedal nerve
F.—foot
F.A.C.P.—first anterocentral pedal nerve
F.G.P.—female genital pore
F.L.P.P.N.—first lateroposterior pedal nerve
F.N.—frontal nerve
GIZ.—gizzard
G.N.—gastric nerve

In a living *L. humilis* the color of the foot (F.) and body normally exposed beyond the rim of the aperture varies from light to dark gray. Occasionally the dorsum of the body is almost black. In a relaxed animal the length of the foot from the tip of the velum (VE.) to the most posterior part is 7.2 mm.; the greatest width at the anterior end is 4.2 mm. The velum (Fig. 2) of *L. humilis* is prominent and the longitudinal mouth as well as part of the dorsal mandible (Fig. 6, D.M.) are on the mid-ventral surface of the velum.

The eyes are located at the base of the triangular tentacles in the anterior angle, and measure about 100 μ in diameter (Figs. 2 and 4). The height from the base of the tentacle to its apex is approximately 0.85 mm. The length of the entire base is 1.9 mm. and the distance between the eyes is about the same.

The male genital pore (Fig. 2, M.G.), situated at the base of the right tentacle in the posterior angle, is a longitudinal slit about 200 μ long.

When the shell is removed, the mantle over the entire body whorl is found to be heavily pigmented but is windowed by circular areas where pigmentation is absent (Fig. 1). These areas vary in size but are generally smaller posteriorly. Through these, the outlines of the organs beneath the mantle may be partly seen (Fig. 4). In the region of the pneumatopore, about 1.70 mm.

H.D.B.—second portion of the hermaphrodite duct
H.D.C.—third portion of the hermaphrodite duct
IN.C.N.—inferior cervical nerve
IN.L.N.—inferior labial nerve
INT.—intestine
I.P.N.—internal pedal nerve
I.PL.N.—internal pallial nerve
L.—liver
L.A.M.—line of attachment of mantle to body
LB.N.—laterobuccal nerve
L.IN.C.N.—lateral inferior cervical nerve
L.L.—lateral lobule
L.M.—longitudinal muscle
L.P.G.—lower prostate gland
L.PL.N.—left pallial nerve
L.R.CO.N.—lateral ramus of the columellar nerve
L.S.C.N.—lateral superior cervical nerve
L.V.N.—lateral velar nerve
MA.—mantle
M.G.—male genital pore
M.IN.C.N.—medial inferior cervical nerve
M.PL.N.—median pallial nerve
M.S.C.N.—medial superior cervical nerve
M.V.N.—medial velar nerve
OE.—esophagus
O.G.—öothecal gland
OP.N.—optic nerve
OT.N.—optotentacular nerve
PE.G.—pedal ganglion
PE.N.—penial nerve
PE.S.—penis sheath
P.G.—parietal ganglion
PL.G.—pleural ganglion
PN.—pneumatopore
PN.L.—pneumatopore lip
P.P.N.—posterior pedal nerve
PRE.—preputium
PRO.M.—protractor muscle of buccal mass
P.V.N.—posterior velar nerve
REN.O.—renal organ
R.M.PRE.—retractor muscle of the preputium
R.M.P.S.—retractor muscle of the penis sheath
R.PL.N.—right pallial nerve
R.P.P.N.—right posterior pedal nerve
S.A.C.P.—second anterocentral pedal nerve
S.C.N.—superior cervical nerve
S.D.—salivary duct
SE.R.—seminal receptacle (Fig. 4)
S.G.—salivary gland
S.L.N.—superior labial nerve
S.L.P.P.N.—second lateroposterior pedal nerve
S.N.—salivary nerve
SPL.N.—splanchnic nerve
S.R.—seminal receptacle (Figs. 10, 13)
S.R.D.—duct of the seminal receptacle
TEN.—tentacle
TE.N.—tentacular nerve
U.P.G.—upper prostate gland
UT.—uterus
VA.—vagina
VB.N.—ventrobuccal nerve
V.D.—vas deferens
VE.—velum
VEN.—ventricle
VL.N.—ventrolabial nerve
VPL.N.—ventropallial nerve

above the edge of the mantle, is a bar of pigment, a constant feature in *L. humilis* found in the region of Ann Arbor (Fig. 1). This bar of pigment runs approximately parallel to the edge of the mantle and is about 2.75 mm. long and 0.75 mm. wide.

The pneumatoporal (or pneumostomal) lip (PN.L.) is under the mantle at the right posterior edge near the tip of the aperture of the shell (Fig. 3). On its lateral surface is the pneumatopore (PN.), the more or less vertical (to the edge of the mantle) opening of the lung; the anus (AN.) is posterior to it. The length of the pneumatoporal lip at its base is 1.5 mm. The pneumatopore is the only opening into the mantle cavity.

The female genital pore (F.G.P.) is a longitudinal slit about 400 μ long under the lip of the pneumatopore (Fig. 12). It is borne on a distinct oval mound of tissue. Occasionally, in sufficiently cleared specimens, the vas deferens is visible as a narrow tube running through the hemocoele wall between the male and female pores.

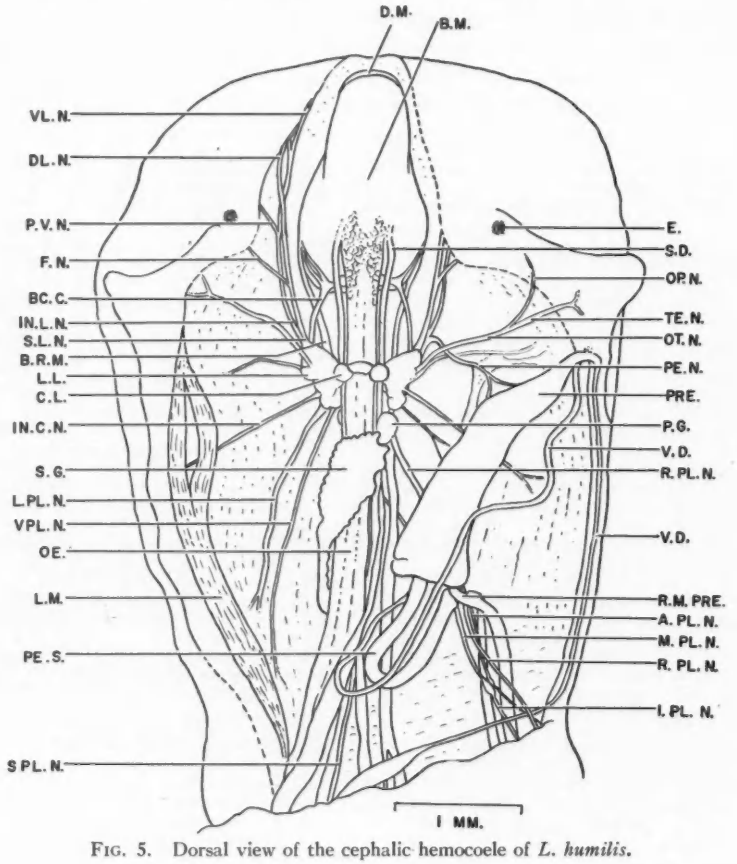


FIG. 5. Dorsal view of the cephalic hemocoele of *L. humilis*.

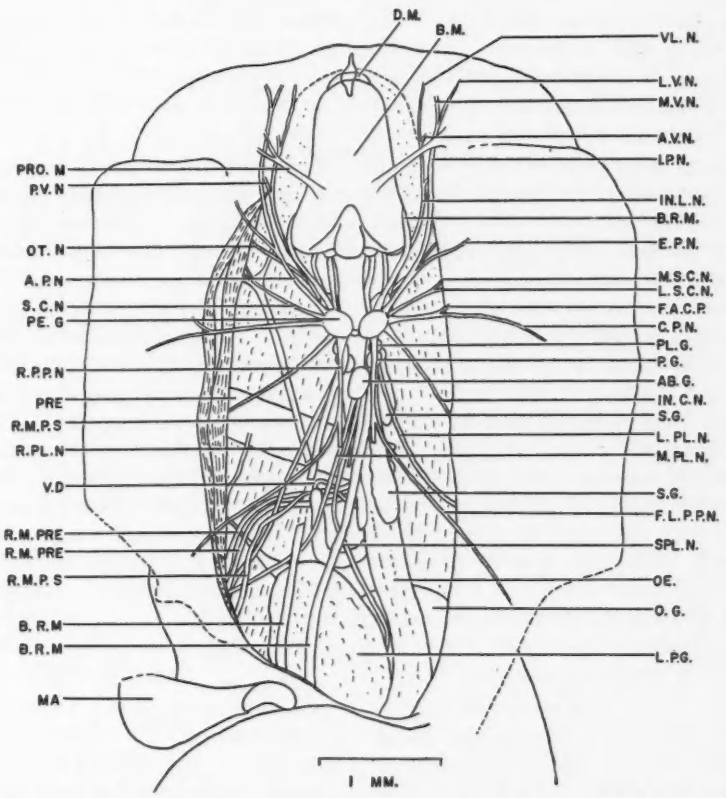


FIG. 6. Ventral view of the cephalic hemocoele of *L. humilis*. Part of each posterior pedal nerve is not shown (beyond the leader to 'M.PL.N.') as well as all of each columellar nerve.

The body cavity of *Lymnaea* has been accurately defined by Carriker (12) and is divided into the cephalic and visceral hemocoeles and the lung. The cephalic hemocoele lies within the head and neck regions. The visceral hemocoele is placed dorsal and posterior to the cephalic hemocoele and ventral to the lung and lies within the shell. The cervical septum, an extension of the diaphragm, separates the cephalic and visceral hemocoeles (12).

Beneath the mantle on the left side the outlines of several organs may be seen (Fig. 4). The distinctness of these outlines varies with the degree of mantle pigmentation. The ventricle (VEN.) and auricle (AUR.) of the heart are usually easily discernible as is the seminal receptacle (SE.R.) which lies mostly under the heart. The seminal receptacle is bright orange in the living snail. The broad, flat, creamy-white kidney or renal organ (REN.O.), posterior to the heart, is attached to and crosses the mantle transversely to the pneumatopore. Its general outline is visible through the mantle. A small extension of the kidney reaches ventrad between the ventricle and the

voluminous albumen gland (ALB.G.). The kidney is exceedingly friable in the living snail, disintegrating into flocculi if manipulated roughly. Dorsal to the albumen gland the outline of part of the uterus (UT.) can be seen. The lung lies dorsal to the uterus and is devoid of accessory respiratory structures as in *Lymnaea stagnalis* (Carriker (12)).

The principal features of the cephalic hemocoele are shown in Figs. 5 and 6. The greatest length of the cephalic hemocoele is approximately 4.5 mm. The floor of the hemocoele is gently rounded from side to side and the posterior portion of the floor narrows and slopes in a posterodorsal direction towards the cervical septum making an angle of about 60 degrees with the ventral surface of the foot. Strong longitudinal muscles (L.M.), which converge posteriorly under the lower prostate (L.P.G.) and öothecal glands (O.G.) to form the columellar muscle, define the walls of this cavity. These muscles extend to both sides of the buccal mass. The expansive arched roof of the cephalic hemocoele extends anteriorly from the line of attachment of the mantle to the body wall. The muscles of the roof arise as a common sheet but soon separate into six broad, flat bands, three on either side of the middorsal line. The posterior pair pass lateroventrad and become continuous with one another on the floor of the cephalic hemocoele where they lie transversely. The central pair extend anterolaterad, and the anterior pair extend anteriad, lateral to the buccal mass. These muscles line the roof of the cephalic hemocoele internally and pass internal to the longitudinal muscles which form the walls of the cephalic hemocoele. Only in well-cleared animals are they visible externally.

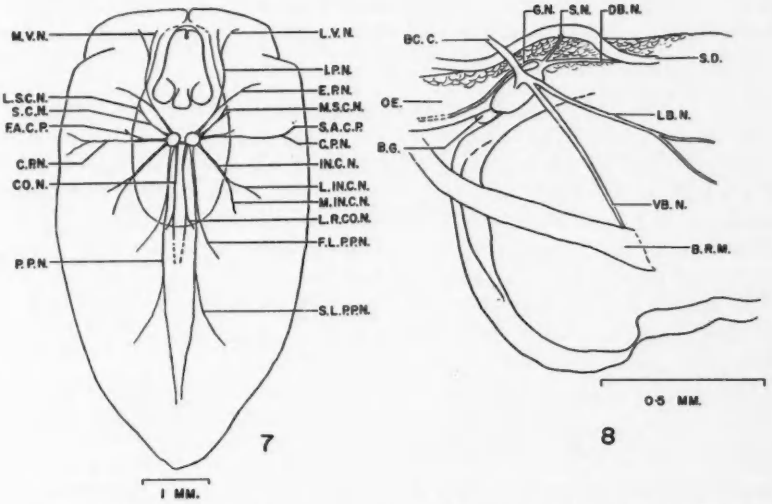
The anterior part of the cephalic hemocoele is occupied by the pear-shaped buccal mass (B.M.). It is a light orange-red in the living snail. The prominent buccal retractors (B.R.M.), which arise in the columellar muscle, insert on the lateral surfaces of the buccal mass. The delicate capitocerebral membrane, which is peppered with pigment, surrounds the buccal mass as it does in *Lymnaea stagnalis* (12), screening this organ from the rest of the cephalic hemocoele. The large preputium (PRE.) occupies the right central portion of the cephalic hemocoele.

Posterior to the ganglia of the central nervous system are the salivary glands (S.G.), which mostly flank the esophagus (OE.) on either side. Two thick salivary ducts (S.D.) lead from the glands into the dorsum of the buccal mass. The esophagus bends slightly to the left in the cephalic hemocoele, penetrates the cervical septum, and turns ventrad, passing beneath the albumen gland. The esophagus is fairly heavily pigmented, especially in its posterior part. It is followed by the crop and the bright, orange-red, bilobed gizzard (Fig. 4, GIZ.), both of which are ventral to the albumen gland. The pylorus, leading from the gizzard, turns anteriad around the right lobe of the gizzard. The alimentary canal continues as the intestine (Figs. 2 and 4, INT.), which turns sharply posteriad, ventral to the esophagus and crop, and follows a course along the left ventral margin of the albumen gland. It then angles sharply to the right and makes a gradual loop upon itself in

the liver (Fig. 2, L.) where it passes superficially into the liver tissue and becomes less distinctly outlined. It continues towards the right along the edge of the mantle beneath the suture of the body whorl. The intestine merges into the short rectum which terminates in the anus (Fig. 3, AN.).

The Nervous System

The central nervous system of *L. humilis* consists of the paired cerebral (C.G.), buccal (B.G.), pedal (PE.G.), pleural (PL.G.), and parietal ganglia (P.G.) and the unpaired abdominal ganglion (AB.G.) (Figs. 5 to 9). The cerebral ganglia lie dorsolateral to the esophagus and are joined by a stout connective; the pedal and parietal ganglia and the abdominal ganglion are ventral to the esophagus. The pleural ganglia are lateral and the buccal ganglia are nestled under the esophagus at its junction with the buccal mass. The buccal ganglia are obscured from view dorsally by the thick salivary ducts but are partially visible from the ventral aspect (Figs. 6 and 8). With exception of the pedal connective, which is delicate and seen only with difficulty, the connectives between the remaining ganglia are stout and conspicuous. The width of the cerebral ganglia and connective, measured from the dorsal aspect, is about one millimeter. The greatest length of a cerebral ganglion is approximately 400 μ and of the abdominal ganglion, 300 μ. The pedal ganglia and the right parietal ganglion, which is markedly larger than the left, are about equal in length to the abdominal ganglion. The remaining ganglia are smaller; the smallest are the buccal ganglia which are about 200 μ in length.



FIGS. 7 and 8. Nerves of the foot and buccal mass of *L. humilis*. FIG. 7. The principal nerves arising from the pedal ganglia and innervating the foot of *L. humilis*. FIG. 8. Lateral view of the main nerves supplying the buccal mass and alimentary canal of *L. humilis*.

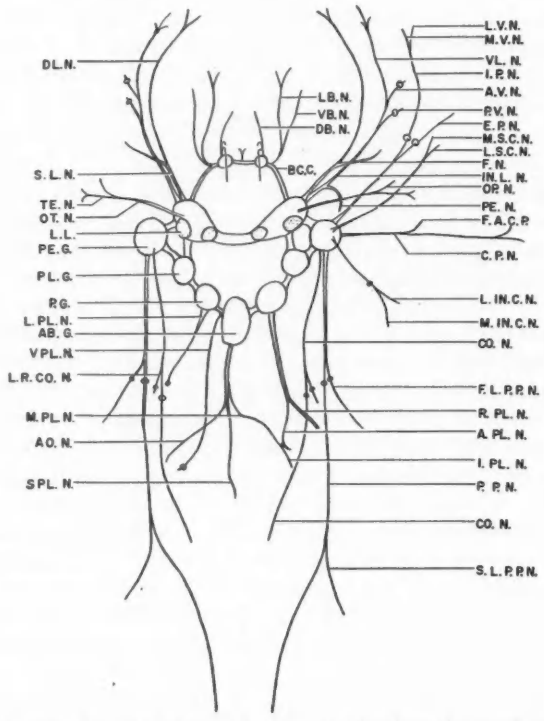


FIG. 9. Diagram of a dorsal view of the nerves arising from the ganglia of the centra nervous system of *L. humilis*. The pedal ganglia are separated and the complete number of pedal nerves is shown for the right pedal ganglion only.

Each cerebral ganglion has two lobules projecting from its surface, the commissural lobule (C.L.) and the lateral lobule (L.L.) (Fig. 5). The lateral lobule is similar to the 'lobule de la sensibilité spéciale' of Lacaze Duthiers (21), which he described for *Lymnaea pereger* and *L. stagnalis*. The superior lobule which Lacaze Duthiers (21) figured for the same two species is not a distinct entity in *L. humilis*. The capitocerebral membrane which envelops the buccal mass surrounds the ganglia of the central nervous system. The nerve trunks passing from them are covered proximally. In *L. humilis* this membrane also surrounds the ganglia internally, as well as externally, separating them from direct contact with the esophagus.

The ganglia of the central nervous system circle the esophagus loosely so that when the buccal mass is retracted by the buccal retractors, the esophagus is able to accommodate accordingly in a posterior direction.

Cerebral Ganglia (Figs. 5, 6, 9)

In *L. humilis* the optic (OP.N.) and tentacular nerves (TE.N.) are branches of a long common trunk, here named the optotentacular nerve (OT.N.). In *L. stagnalis* this common trunk is short (12). The optotentacular nerve

arises close to the lateral lobule of each cerebral ganglion and divides into the delicate optic nerve and the posterior, thicker tentacular nerve. The optic nerve can be traced to the eye with relative ease. It passes into the anterior muscles of the roof of the cephalic hemocoele. As it enters the base of the tentacle, the tentacular nerve divides into anterior and posterior branches. Occasionally the anterior branch sends off a small nerve near the point of division of the two main branches.

The superior labial nerve (S.L.N.) arises dorsal to the inferior labial nerve (IN.L.N.) and gives rise to the frontal nerve (F.N.) and then passes superficially into the hemocoele wall lateral to the buccal mass as the dorsolabial nerve (DL.N.), which divides into two branches terminally. The inferior labial nerve gives rise to the anterior and posterior velar nerves (A.V.N., P.V.N.) and continues as the ventrolabial (VL.N.), which terminates ventrolateral to the dorsal mandible in a slight bulb. At this point it branches into three delicate nerves that can be traced only a very short distance. For the most part, the ventrolabial nerve passes only superficially into the wall of the cephalic hemocoele. The superior labial nerve generally runs dorsal to the longitudinal muscles of the wall of the hemocoele and the inferior labial generally ventral to them.

The origin of the velar nerves of *L. humilis* varies and this has been particularly observed on the right side. The anterior and posterior velar nerves may arise as separate nerves from the inferior labial, the anterior arising at a level midway between the anterior and posterior ends of the buccal mass, the posterior at a level near the junction of the esophagus and the buccal mass. The nerve may also arise from a common trunk which divides immediately. This trunk is usually located near the junction of the esophagus and buccal mass.

The buccocerebral connectives (BC.C.) arise ventral to the inferior labial nerve uniting the buccal and cerebral ganglia.

The penial nerve (PE.N.) is a branch of the right inferior labial nerve and innervates the preputium branching several times. A small branch of the penial nerve passes to the anterior penial retractor muscle.

Buccal Ganglia (Figs. 8, 9)

These ganglia are joined by the small buccal connective, and send out the principal nerves innervating the greater part of the alimentary canal (12). The nerve supply of the alimentary canal has been thoroughly worked out for *L. stagnalis* by Carriker (12). As in *L. stagnalis* six main nerve trunks arise from these ganglia: the paired ventrobuccal (VB.N.), laterobuccal (LB.N.), dorsobuccal (DB.N.), salivary (S.N.), and gastric nerves (G.N.) and the unpaired postbuccal nerve arising from the buccal connective (Fig. 8). Their distribution, as far as could be traced, is the same as in *L. stagnalis*.

Pleural Ganglia (Figs. 6, 9)

These ganglia give rise to no nerves.

Parietal Ganglia (Figs. 5, 6, 9)

The left pallial nerve (L.PL.N.), the only nerve arising from the left ganglion, passes posterodorsad and penetrates the posterior part of the roof of the cephalic hemocoele where it divides twice and occasionally three times. The right pallial nerve (R.PL.N.), which is the stoutest nerve of the central nervous system of *L. humilis*, also passes in a posterior direction dorsal to the vagina, the duct of the seminal receptacle, and the vas deferens (Figs. 5 and 6). The anterior pallial nerve (A.PL.N.) arises ventral to the right pallial and remains within a common sheath surrounding both these nerves to near the point where the anterior pallial nerve connects with the median pallial nerve (M.PL.N.).

Abdominal Ganglion (Figs. 6, 9)

The ventropallial nerve (VPL.N.) arises from the left side of the abdominal ganglion and passes back beneath the esophagus through the cervical septum and penetrates the longitudinal muscles of the left side near their junction with those of the right (to form the columellar muscle) (Figs. 5 and 9).

The splanchnic nerve (SPL.N.) arises dorsal to the median pallial nerve and follows a course and gives rise to branches in a manner similar to *L. stagnalis* (12) (Figs. 5, 6, and 9). The median pallial nerve from the abdominal ganglion unites with the anterior pallial from the right parietal ganglion and forms the internal pallial nerve (I.PL.N.), which passes ventral to the vas deferens and vagina (Figs. 5 and 9). Where the median pallial and anterior pallial nerves unite there is a small swelling, and a delicate nerve arises from it and passes to the body wall. The aortic nerve (AO.N.), a small and delicate nerve, follows a course similar to *L. stagnalis* (12) and passes along the cephalic artery (Fig. 9).

Elo (15) figured four nerves arising from the abdominal ganglion,—an intestinal nerve, a genital nerve, a cutaneous pallial nerve, and an anal nerve. The intestinal nerve is apparently the splanchnic nerve of Carriker (12) and is recognized as such here. Elo (15) renamed the aortic nerve (which remains unchanged in Carrikers' (12) work) the genital nerve because he maintained that it supplied the seminal receptacle. As far as could be determined, the aortic nerve does not innervate the seminal receptacle of *L. humilis*. According to Elo (15) the cutaneous pallial nerve serves a small region of the mantle. A corresponding nerve was not found in *L. humilis*. The anal nerve of Elo appears to be the median pallial nerve of Carriker (12) and the present study.

Pedal Ganglia (Figs. 6, 7, 9)

The paired pedal ganglia of *L. humilis* function primarily in the control of locomotion. The innervation of the foot of lymnaeid snails has not received as much attention as other structures of these snails. Lacaze Duthiers (21) and Elo (15) described the main trunks of the pedal ganglia (the former author in *L. stagnalis* and *L. pereger*; the latter in *L. stagnalis* only) but do not follow their detailed ramifications. The pedal nerves were not dealt

with by Carriker (12). Both Lacaze Duthiers (21) and Elo (15) described six pairs of nerves arising from the pedal ganglia of *L. stagnalis*: the columellar nerves, superior cervical nerves, inferior cervical nerves, and the superior pedal, inferior pedal, and intermediate or median pedal nerves. The superior, inferior, and median pedal nerves are better named the anterior (A.P.N.), posterior (P.P.N.), and central pedal nerves (C.P.N.) because they are primarily oriented in an anteroposterior direction in the foot (Figs. 7 and 9). Schmalz (22) described 15 pairs of nerves arising from the pedal ganglia of *H. pomatia.*

Elo (15) homologized the pedal nerves of *H. pomatia* and *L. stagnalis* in the following way:

Lymnaea stagnalis	*Helix pomatia*
N. cervicalis superior	*N. cutaneus pedalis primus*
N. cervicalis inferior	*N. cutaneus pedalis secundus*
N. columellaris	*N. cutaneus pedalis tertius*
N. pedalis superior, medius, and inferior	*N. musculi pedali* I–XI

The 'nervus arteriae pedalis anterior' and 'posterior' of Schmalz (22) were not homologized by Elo (15).

In this study an attempt has been made to trace the innervation of the foot of *L. humilis* as far as possible. The paired pedal ganglia of *L. humilis* each give rise to six main nerve trunks; the anterior (A.P.N.), central (C.P.N.), and posterior pedal nerves (P.P.N.); the superior (S.C.N.) and inferior cervical nerves (IN.C.N.), and the columellar nerve (Figs. 7 and 9). In the present study, 13 newly named or described branches of these six main trunks are given.

The anterior pedal nerve arises from the ventral, anterior portion of the pedal ganglion and proceeds anteroventrad dividing immediately posterior to the buccal mass into the internal (I.P.N.) and external pedal nerves (E.P.N.). Both these nerves pass into the floor of the hemocoele lateral to the buccal mass. The internal pedal nerve divides into two nerves of approximately equal size, the medial (M.V.N.) and lateral velar nerves (L.V.N.), which pass into the velum. The anterior pedal nerve is ventral to the inferior labial nerve.

The central pedal nerve takes its origin from a ventrolateral aspect, and, near the wall of the hemocoele sends a small branch, the first anterocentral pedal nerve (F.A.C.P.), through the floor of the hemocoele into the foot. The central pedal continues laterad and gives rise to a second nerve which passes anteriad, the second anterocentral pedal nerve (S.A.C.P.). Turning slightly posteriad, the central pedal continues almost to the edge of the foot.

From its ventroposterior origin, the stout posterior pedal nerve passes directly posteriad and disappears into the posterior part of the floor of the cephalic hemocoele. Before disappearing, a lateral branch arises from it, the first lateroposterior pedal nerve (F.L.P.P.N.). This nerve curves laterad. The posterior pedal nerve continues posteriad and a second nerve, the second

lateroposterior pedal nerve (S.L.P.P.N.), arises from it and proceeds in a manner similar to the first. Beyond the second lateroposterior pedal nerve, the posterior pedal curves slightly mesiad and then disappears in the tissues of the foot. Throughout its course, the posterior pedal runs approximately parallel to the ventral surface of the foot.

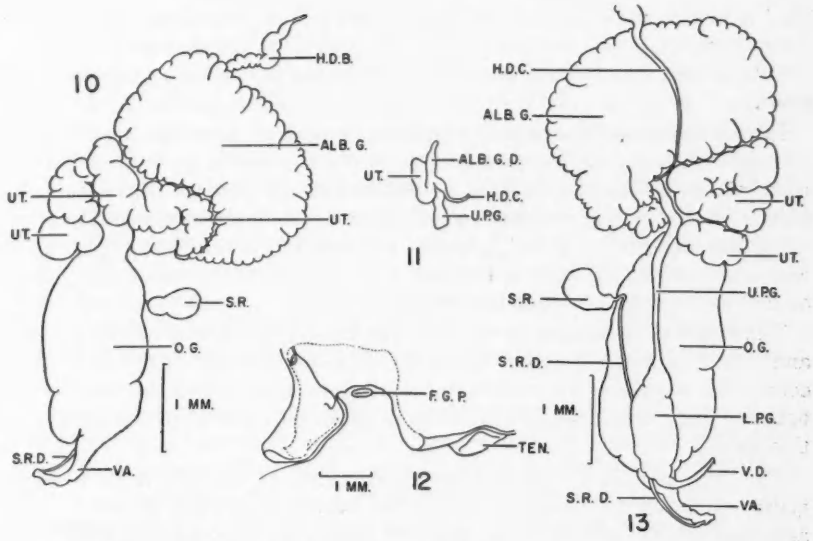
The superior cervical nerve arises between and dorsal to the anterior and central pedal nerves and divides into two nerves, the medial (M.S.C.N.) and lateral superior cervical nerves (L.S.C.N.), which penetrate the longitudinal muscles of the cephalic hemocoele wall through a distinct elongate slit (Figs. 6, 7, and 9). The superior cervical nerve passes laterad and anterodorsad.

The inferior cervical nerve originates near the pleuropedal connective and also penetrates the longitudinal musculature through a conspicuous longitudinal slit. It runs posterolaterad. Lacaze Duthiers (21) described this nerve in *L. stagnalis* as furnishing branches which extend as far as the junction of the mantle with the body. Immediately after leaving the cephalic hemocoele, the inferior cervical nerve divides into the medial (M.IN.C.N.) and lateral inferior cervical nerves (L.IN.C.N.) (Figs. 7 and 9.)

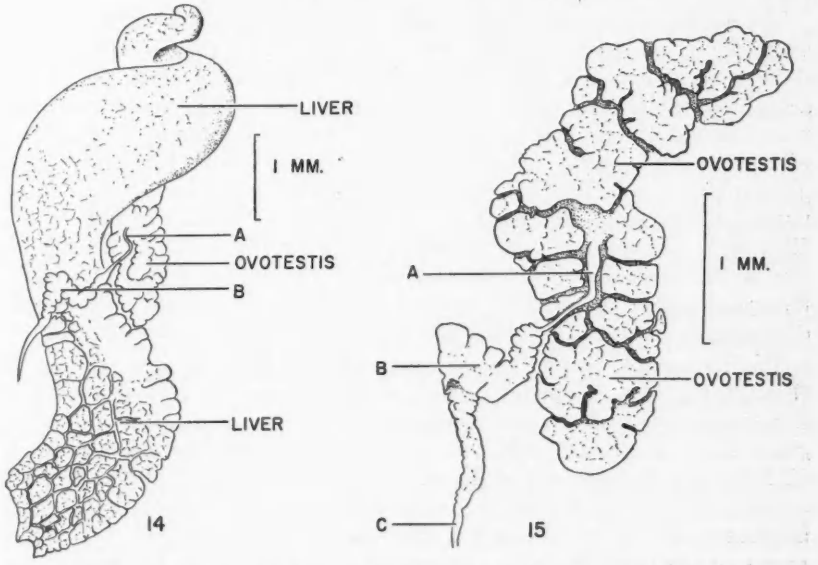
The columellar nerve (CO.N.) arises dorsomedial to the posterior pedal and disappears into the musculature of the posterior wall of the hemocoele. It gives rise to a small lateral branch, the lateral ramus of the columellar nerve (L.R.CO.N.). The general distribution of the columellar nerve of *L. humilis* agrees with that found by Lacaze Duthiers (21) in *L. stagnalis* and *L. pereger*. The columellar nerve described here must not be confused with the columellar nerve of Carriker (12). This columellar nerve is a branch of the splanchnic nerve (which arises from the abdominal ganglion) and passes over the columellar muscle (12). However, the paired columellar nerves of Lacaze Duthiers (21) are appropriately named for they pass into the musculature of the posterior wall of the cephalic hemocoele of *L. humilis* at the point where the longitudinal muscles of the lateral walls of the cephalic hemocoele converge to form the columellar muscle.

Reproductive System

As in other Lymnaeidae (14, 17, 9) the reproductive system of *L. humilis* is divided into three portions: (1) the ovotestis and its duct, (2) the female system, and (3) the male system (Figs. 10 to 15). The female system consists of the oviduct, which is much reduced in *L. humilis*, the uterus (UT.), the öothecal gland (O.G.) of Holm (17), and the vagina (VA.). The mass membrane gland of Crabb (14) constitutes part of the öothecal gland of Holm (17). Described as accessory glands in *L. stagnalis* by Holm (17) are the albumen gland (ALB.G.), which is very large in *L. humilis*, the muciparous gland, and the seminal receptacle (S.R.). The muciparous gland is the egg membrane gland of Crabb (14) and the nidamental gland of older authors. The muciparous gland is not a distinct entity in the gross morphology of *L. humilis*. The seminal receptacle is closely associated with the left posterior portion of the öothecal gland and is visible topographically under the heart (Fig. 4).



FIGS. 10–13. The reproductive system of *L. humilis*. FIG. 10. Dorsal view of the reproductive system. FIG. 11. The junction of the uterus, albumen gland duct, third portion of the hermaphrodite duct, and upper prostate gland. FIG. 12. The location of the female genital pore. FIG. 13. Ventral view of the reproductive system.



FIGS. 14–15. The reproductive system of *L. humilis*. FIG. 14. The ovotestis partly dissected from the liver. FIG. 15. The hermaphrodite duct, and ovotestis, showing its lobulated structure.

The principal structures of the male reproductive tract are the prostate (U.P.G., L.P.G.), the vas deferens (V.D.), and the large copulatory organ (PRE., PE.S., Figs. 5 and 6). The vas efferens is considered absent in *L. humilis*.

In reproductively active snails the female system is by far the larger of the two systems and completely obscures the prostate from dorsal view. Most of the prostate is confined to the mid-ventral surface of the öothecal gland. As in *L. stagnalis* the two systems are closely apposed to one another along their length except at their external outlets. The öothecal gland and part of the uterus are more or less centrally located but the remaining portion of the female tract is on the left side of the body.

The öothecal gland is directed from the left side toward the right tentacle and makes an angle of about 35 degrees with the left edge of the foot. The columellar muscle is approximately ventral to the anterior portion of the öothecal gland but gradually is situated under the right posterior portion of this gland.

The ovotestis (or hermaphrodite gland) (Figs. 14 and 15) is an elongate gland, embedded in the columellar surface of the liver. It is yellow in color and externally it is irregularly lobulated into about 25 lobules. In *L. stagnalis* it is said to be a dichotomously lobulated sac composed of about 100 lobules which are separated from the liver lobules by loose connective tissue and blood spaces (4). The ovotestis begins about the middle of the second whorl. It is dissected out of the liver with relative ease.

The hermaphrodite duct arises from the central columellar surface of the ovotestis; in live specimens it is yellow in color. This duct is divided into three distinct parts (Fig. 15). The first (A.), a portion of which lies within a short but rather deep sulcus in the ovotestis, is narrow and tapers gradually to the second or vesicular part. In relaxed and fixed material it is usually elbowed. The transition between the first and second parts is abrupt. This portion (B.) is wide and flat and is usually looped upon itself two or three times. It has about 20 diverticula, which, because they are almost always filled with spermatozoa, are sometimes called seminal vesicles. The vesicular portion merges into the third division (C.) which is a long narrow tube that passes under the ventral surface of the albumen gland and turns dorsad disappearing between this gland and the uterus–öothecal gland complex.

The hermaphrodite duct becomes very fine and hair-like at its distal end (Fig. 13, H.D.C.). Repeated dissections have shown that it terminates in a small bulbous expansion which joins with the short, stout, pigmented albumen gland duct (ALB.G.D.), which in turn is united with the upper prostate (U.P.G.) and the beginning of the uterus (UT.) (Fig. 11). This arrangement is quite different from that in *L. stagnalis* where the hermaphrodite duct bifurcates into an oviduct, and a thin, short, vas efferens (14, 17), which passes to the prostate gland. According to Holm (17) in *L. stagnalis* the albumen gland duct passes into a translucent finger-like vesicle which opens at its base into the coiled uterus. The oviduct passes dorsally around the

base of this vesicle and is divided into two branches. One opens into the cavity of this vesicle and the other opens into the uterus. The small expansion at the end of the hermaphrodite duct in *L. humilis* may represent a fusion of the divided oviduct and vas efferens of *L. stagnalis*. In *L. humilis*, there is no evidence of a vesicle to which the albumen gland duct is connected as in *L. stagnalis*. Such structural consolidation is understandable in *L. humilis* since it is usually not more than one quarter the length of *L. stagnalis*.

The uterus is a white, loosely-coiled, saccular structure which surrounds the posterior portion of the öothecal gland and upper prostate. A membrane stretches between the coils of the uterus making it difficult to uncoil it. In gross dissection, there is no evidence of a muciparous gland which can be distinguished from the saccular uterus as in *L. stagnalis* (17). According to Holm (17) the muciparous gland functions to embed eggs in a mucous matrix within the egg mass.

The uterus merges into the öothecal gland, which, with the exception of a very short, upper narrow portion, is a stout organ in *L. humilis* whose sides are more or less parallel. In *L. stagnalis* the upper portion of the öothecal gland is a translucent cylindrical tube of some length while the lower portion is pear-shaped (14, 17). Transverse striae are present externally on this gland, as in *L. stagnalis*.

The vagina (VA.) arises from an elongate promontory on the anteroventral surface of the öothecal gland. The vagina is relatively broad and opens through the right wall of the cephalic hemocoele (Fig. 12). The position and shape of the pore have already been described. Where the duct of the seminal receptacle (S.R.D.) passes into the anterior face of the vagina there is usually a slight swelling. The duct of the seminal receptacle passes diagonally across the ventral surface of the öothecal gland and is partly obscured ventrally by the lower prostate. The duct is thread-like and the seminal receptacle is pear-shaped and a bright orange in the living snail.

The narrow, upper portion of the prostate (U.P.G.) is about twice the length of the lower bulbous part (L.P.G.). The upper prostate is yellow and the lower white in the living snail. The upper region is a long oval or flat tube which tapers somewhat as it merges into the lower portion which is occasionally heavily pigmented.

The vas deferens (V.D.) is broad at first but gradually narrows as it approaches the right body wall, which it penetrates anterior to the vagina (Fig. 5). It slopes slightly ventrad within the wall of the cephalic hemocoele and emerges slightly posterior to the male genital pore and follows a winding course until it continues into the penis sheath. Proportionately, it is shorter than that of *L. stagnalis*.

The penis sheath (Fig. 5, PE.S.) is about equal in length to the muscular preputium (PRE.). The preputium is always broad and square-shouldered where it receives the penis sheath. Near the genital pore several bands of muscle act as protractors of the preputium and a similar group near the penis sheath serve as retractors. This arrangement is similar to that in *L. stagnalis* (17).

Acknowledgments

It is a pleasure to acknowledge the advice and criticism of Dr. Henry van der Schalie of the University of Michigan throughout this study. I am grateful to Dr. T. L. Jones, Principal of the Ontario Veterinary College, for his support of this study. I also wish to express my appreciation to Dr. Rodger D. Mitchell of the University of Vermont for his many helpful suggestions.

References

1. ABDEL-MALEK, E. T. Menthol relaxation of helminths before fixation. J. Parasitol. **37**, 321 (1951).
2. ABDEL-MALEK, E. T. Morphological studies on the family Planorbidae (Mollusca: Pulmonata). I. Genital organs of *Helisoma trivolvis* (Say) (Subfamily Helisomatinae F. C. Baker, 1945). Trans. Am. Microscop. Soc. **73**, 103–124 (1954).
3. ABDEL-MALEK, E. T. Morphological studies of the family Planorbidae (Mollusca: Pulmonata). II. The genital organs of *Biomphalaria boissyi* (Subfamily Planorbinae, H. A. Pilsbry, 1934). Trans. Am. Microscop. Soc. **73**, 285–296 (1954).
4. ARCHIE, V. E. The histology and developmental history of the ovotestis of *Lymnaea stagnalis*. Unpublished thesis, University of Wisconsin. 1942.
5. BAKER, F. C. The gross anatomy of *Limnaea emarginata* variety *mighelsi* Binney. Bull. Chicago Acad. Sci. **2**, 191-211 (1900).
6. BAKER, F. C. The Lymnaeidae of North and Middle America, recent and fossil. Chicago Acad. Sci., Spec. Publ. No. 3, i-xvi, 1–539 (1911).
7. BAKER, F. C. The fresh water Mollusca of Winsconsin. Part 1. The Gastropoda. Wisconsin Geol. and Nat. Hist. Surv. Bull. 70, i-xx, 1–507 (1928).
8. BAKER, F. C. The molluscan family Planorbidae. University Illinois Press, 530. Collation, revision and additions by H. J. van Cleave (1945).
9. BAKER, H. B. Anatomy of *Lanx*, a limpet-like lymnaeid mollusk. Proc. Calif. Acad. Sci. **14**, 143–169 (1925).
10. BARGMANN, H. E. The morphology of the central nervous system in the Gastropoda Pulmonata. J. Linnean Soc. London (Zool.) **37**, 1–59 (1930).
11. BAUDELOT, M. L'appareil générateur des mollusques gasteropodes. Ann. Sci. Nat., Ser. IV, **19**, 135–222 (1863).
12. CARRIKER, M. R. Morphology of the alimentary system of the snail *Lymnaea stagnalis appressa* Say. Trans. Wisconsin Acad. Sci. **38**, 1–88 (1946).
13. CARRIKER, M. R. and Bilstad, N. M. Histology of the alimentary system of the snail *Lymnaea stagnalis appressa* Say. Trans. Am. Microscop. Soc. **65**, 250–275 (1946).
14. CRABB, E. D. Anatomy and function of the reproductive system in the snail *Lymnaea stagnalis appressa* Say. Biol. Bull. **53**, 55–66 (1927).
15. ELO, J. E. Das Nervensystem von *Limnaea stagnalis* (L.) Lam. Ann. Zool. Soc. Zool. Botan. Fennicae Vanamo. **6**, 1–40 (1938).
16. FRASER, L. A. The embryology of the reproductive tract of *Lymnaea stagnalis appressa* Say. Trans. Am. Microscop. Soc. **65**, 279–298 (1946).
17. HOLM, L. W. Histological and functional studies on the genital tract of *Lymnaea stagnalis appressa* Say. Trans. Am. Microscop. Soc. **65**, 45–68 (1946).
18. HUBENDICK, B. Lymnaeidae, their variation, morphology, nomenclature, and distribution. Kgl. Svenska Vetenskapsakad. Handl. **3**, 1–223 (1951).
19. HUBENDICK, B. Phylogeny in the Planorbidae. Trans. Zool. Soc. London. **28**, 453–542 (1955).
20. KLOTZ, J. Beitrag zur Entwicklungs-geschichte und Anatomie des Geschlechtsapparates von Lymnaeus. Jena. Z. Naturw. **23**, 1–40 (1889).
21. LACAZE DUTHIERS, H. DE. Du système nervoux des mollusques gastéropodes pulmonés aquatiques et d'un nouvel organe d'innervation. Arch. zool. exptl. et gén. **1**, 437–500 (1872).
22. SCHMALZ, E. Zur morphologie des Nervensystems von *Helix pomatia* L. Z. wiss. Zool. **111**, 506–568 (1914).

RADIOPHOSPHORUS METABOLISM IN ZOOPLANKTON AND MICROORGANISMS[1]

EUGENE HARRIS[2]

Abstract

The exchange of radiophosphorus between water on the one hand and *Artemia*, *Gammarus*, and microorganisms on the other was observed. Results are expressed as turnover time, which is related to the rate at which phosphorus moves back and forth, being defined as the time required for as much material as is present to enter or leave a phase of the system. T_t for *Artemia* is 14 hours; for *Gammarus* 43 hours; the equilibrium between organic and inorganic phosphorus in the presence of microorganisms has a T_t of 19 hours. In *Gammarus* there is a lag period of a few hours before they reach a maximal uptake rate. *Gammarus*, treated with terramycin to kill bacteria, do not remove appreciable quantities of radiophosphorus from water. Evidently they must acquire their phosphorus from particulate matter, such as bacteria.

Coffin *et al.* (2) added radiophosphorus to the Punchbowl, a small acid bog lake west of Halifax. It was found that the ratio of the maximum count per wet weight of zooplankton to maximum count for the same quantity of lake water was 40,000. A comparison of the rates of turnover of ^{32}P showed that algae and sponges equilibrated most rapidly, from 40 to 50 times faster than zooplankton. However, zooplankton did take up large quantities of ^{32}P surprisingly quickly after its addition to the water. This raised the question of the mechanism of ^{32}P absorption by zooplankton. Do they obtain a large quantity of their phosphorus by direct absorption through their body walls, gills, and intestines, or must they obtain it in organic combination in their food? In the present paper an attempt is made to answer this question.

A striking feature of limnology is the small amount of reliable data on the quantitative part played by bacteria in lake metabolism. A summary of our knowledge of bacteria as food for other organisms is found in Welch (17). He says, "Many of the Protista (Protophyta and Protozoa) devour bacteria. The same seems true of the metazoan mud feeders and of some of the higher plankton organisms. Beyond these bare facts, far too little is known about this relation in water. Few quantitative data are available".

The addition of radioactive phosphorus, ^{32}P, to lake waters has shown that a lake is not, as one might think, a body of water containing nutrients but an equilibrated system of water and solids, in which, under ordinary conditions, most of the nutrients are in the solids (Hayes *et al.* (5)). These authors have set up an equation to describe the phosphorus equilibrium between water and solids, from which the turnover times of the two phases

[1] Manuscript received September 9, 1957.
Contribution from the Zoological Laboratory, Dalhousie University, Halifax, N.S. With financial assistance from the National Research Council of Canada and the Nova Scotia Research Foundation.

[2] This paper is the substance of a Master's thesis by the late Eugene Harris, who left Dalhousie for doctoral studies at Yale University, where, in his final year, he died of cancer. His scientific promise was great and he was regarded with affection by this fellow students, who have established a prize in his honor at Dalhousie.

are derived. Turnover times are related to the rate at which phosphorus moves back and forth between the solids and water. For water the turnover time ranged from a few days to a month in several lakes tested, and the former was 5 to 10 times that of water. The experiments described below were laboratory tests on several aquatic organisms and from them the turnover times for these "solids" are calculated. Radiophosphorus was added to water and its uptake by the living material was followed, being described by the equation

$$Ac = Ac_\infty (1 - e^{-t/T})$$

where Ac is the activity (counts of ^{32}P per g. fresh weight per minute) at any time, t, and Ac_∞ is the final activity reached. T is a number which is sought as an intermediary on the way to the estimation of the turnover time, T_t. In practice a straight line is obtained when $\log(Ac_\infty - Ac)$ is plotted against time, the equation being

$$\log(Ac_\infty - Ac) = \log Ac_\infty - (0.4343\ t/T).$$

An example of the solution for T_t is given in a later section.

Materials and Methods

The brine shrimp, *Artemia*, was used in the first experiment because of the anticipated ease with which it may be cultured, and its close resemblance in size and feeding habits to many crustaceans found in lakes. The animals were reared from eggs, hatched in Halifax sea water, and fed on yeast, supplemented in later cultures by a little *Ulva* or *Chlamydomonas*.

Another test animal was the familiar seashore sandhopper or beach flea, *Gammarus locusta*, which was collected under stones and in seaweed in the North West Arm, Halifax. It is an amphipod crustacean. Specimens were kept in a cold room at about 4° C. in a slanted aquarium so one end was out of the water. With only a little mud, stones, and fragments of seaweeds in the aquarium, the animals live 2 or 3 months without attention.

Experiments were also conducted with the fresh-water gammarid, *Hyalella*, collected from lakes. It was found, however, that cannibalism was common among the specimens in the test beakers, and this made it impossible to get reliable results on phosphorus exchange with the water. Results on this animal are therefore omitted.

Artemia was probably more suitable than marine *Gammarus* for the experiment because of its smaller size (12 mm. long) and its continuous movement and filtering of food from the water. *Gammarus* would lie quiescent for long periods. In addition, *Gammarus* is a littoral genus accustomed to spending the low tide intervals out of water.

Upon removal of the specimens from the radioactive solution, excess water was immediately removed from them with filter paper, and they were rapidly weighed. They were then frozen with liquid carbon dioxide. Extraction of

acid soluble phosphorus was carried out in a refrigeration room at 3°–5° C. The method used was similar to that of McCarter and Steljes (12) based on that of LePage and Umbreit. Frozen specimens were ground to a powder with agate or glass mortar and pestle. One to two milliliters of 10% trichloroacetic acid was added to the powder and ground to form a homogenate. A medicine dropper was used to transfer the homogenate to a 12 ml. centrifuge tube. The mortar, pestle, and medicine dropper were washed successively with small portions of the trichloroacetic acid which were added to the centrifuge tube until it reached the 10 ml. mark. The homogenate was centrifuged and the supernatant fluid transferred to a 50 ml. volumetric flask. In order to wash the residue it was suspended three times in 10-ml. portions of trichloroacetic acid, the supernatants being added to the volumetric flask. Finally the volumetric flask was made up to the mark with trichloroacetic acid.

The residue was dissolved in 5 ml. of concentrated nitric acid with gentle heating. It was then transferred to a 100 ml. Kjeldahl flask to be digested. Whole animals and aliquots of the trichloroacetic acid extract were also digested in Kjeldahl flasks. Digestion was carried out using a modification of King's method (8). After partial digestion with concentrated nitric acid the flask was allowed to cool slightly, 0.2 ml. of 72% perchloric acid was added, and digestion was completed. After the flask had cooled, its contents were quantitatively transferred to a 25 ml. volumetric flask made up with distilled water.

Inorganic radioactive phosphorus was determined by a method developed by McCarter and Steljes (12). Ten milliliters of the trichloroacetic acid extract were transferred to a 40 ml. conical centrifuge tube. A sufficient volume of a solution of potassium dihydrogen phosphate was added to bring the total ^{31}P in the sample up to 1.0–1.2 mg. and then 1 ml. of concentrated perchloric acid and 2 ml. of 50% saturated ammonium nitrate were added. The mixture was stirred by agitating the tube, which was then placed in a water bath at 80° C. for 10 minutes. Five milliliters of 5% molybdic acid reagent of Willard and Diehl (19) was added slowly with agitation. The tube was allowed to stand at room temperature for 1 to 2 hours with occasional mixing. A small portion of the precipitate was found to float on the surface of the liquid and this was sunk with aerosol or ethyl alcohol. After the precipitate had been collected by centrifugation it was washed twice in 10 ml. portions of 1 : 50 nitric acid, the supernatant liquids being discarded. For collection of the precipitate, disks of Whatman No. 50 filter paper were ringed with a wax pencil to prevent precipitate from creeping over the edges. The disks were placed on a sintered glass filter and the precipitate collected by suction as the suspension was dropped on from a medicine dropper. The precipitate was washed with ethyl alcohol followed by a dilute solution of a synthetic resin, Hypalon P-4, dissolved in acetone. The disk was glued to a counting tray with rubber cement. Tests showed that no correction for self-absorption of radiation was necessary.

Radiophosphorus was obtained from the Chalk River Laboratory in the form of carrier-free phosphate. One milliliter lots containing 1 mc. ^{32}P were diluted to 100 ml. and, in order to prevent bacterial growth in the solution, were kept overnight in an oven at 95° C. Each beaker in an experiment contained about 0.001 mc. of the ^{32}P and at no time did the animals show harmful effects from the radioactivity.

Samples were prepared for measurement of their activity by evaporation of 1 ml. duplicates of the test solution in a counting tray. The activity of the samples was determined with a counter and scaler as described by Hayes and Jodrey (4).

First attempts to rid *Gammarus* of bacteria were made by using an antiseptic, dettol (*p*-chloro-*m*-xylenol). The concentration used was 1 ml. per liter of autoclaved sea water and the contact periods 5 to 35 minutes. The bacterial count was reduced considerably, but the method also proved deleterious to the animals.

Gammarus were made bacteria free by use of terramycin hydrochloride, which was not harmful to the animals. Before the animals were placed in contact with the antibiotic they were washed in sterile sea water. Antibiotics are not effective against molds and actinomycetes, but the washing procedure prior to treatment reduces their numbers considerably. The concentrations of terramycin used were 250 mg. and 100 mg. per 100 ml. of water. The animals were kept in contact with the terramycin from 1 to 2 hours. They were then removed from the solution with sterile glass tubes as described by Stuart *et al.* (16), and taken through a sterile sea water bath in order to wash off the terramycin before use in a ^{32}P test.

Checks were made for bacterial growth in each experimental flask as the animals were removed from it. One milliliter of the sea water from the flask was plated in a beef–peptone sea water agar modified after ZoBell (20). Counts were made 1 week after plating. No flask was completely free of bacteria but a few contained as low as five colonies per milliliter in the controls. Spencer used penicillin and streptomycin in order to obtain bacteria free diatoms (15). He did not get his cultures completely free of bacteria even when he used a combination of the two antibiotics.

Uptake of Radiophosphorus by Microorganisms

While making a routine check on the accuracy of the method for determining inorganic radiophosphorus in distilled water, it was discovered that only 40% of the total ^{32}P was precipitated by the method as above described. When some of the water was ashed with nitric and perchloric acids, over 99% of the ^{32}P was recovered. This means that microorganisms (yeasts, molds, bacteria, etc.) had converted three-fifths of the total phosphorus into the organic form. This is a rather remarkable phenomenon in view of the fact that the sample was carrier free, so that there was no phosphorus to support life except the few labelled atoms. Dilution was made with distilled water,

so the only other substance present was 1 ml. of dilute HCl in which the ^{32}P was delivered. The nutrient supply seems very scanty to permit so much evidence of life.

The conversion to organic ^{32}P was further studied on a jar of water, collected from Chocolate Lake near Halifax. After the water had stood for 24 hours, it was filtered, ^{32}P added, and it was stirred gently in a dark room. Both total and inorganic ^{32}P were estimated with results as shown in Fig. 1. As a check, two autoclaved solutions of radiophosphorus were kept under sterile conditions and were tested for loss of ^{32}P on the 8th and 13th days. There was no loss from the water in either flask.

The top curve of Fig. 1 shows that after 155 hours 16.7% of the ^{32}P had disappeared from the water, a loss credited to sessile bacteria growing on the walls of the container. Norman Beckett in this laboratory had already observed a similar phenomenon in unfiltered lake water kept in a jar, in room light. After 10 days 45% of the ^{32}P had disappeared, a portion perhaps to algal cells on the walls, and to dead microorganisms settling to the bottom.

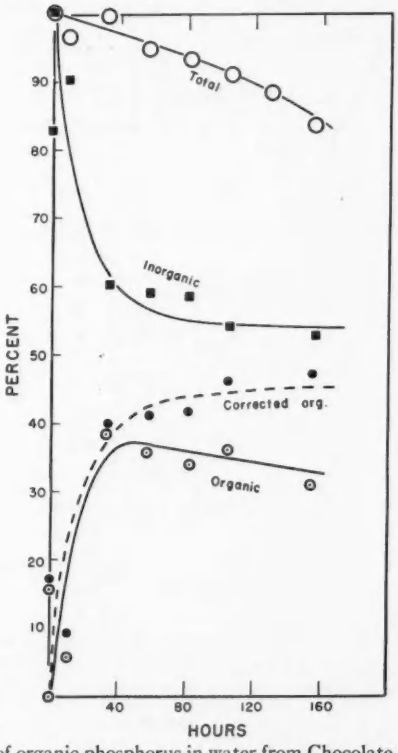


FIG. 1. Synthesis of organic phosphorus in water from Chocolate Lake, N.S. Prior to the test, water was passed through ordinary filter paper (not molecular filter). The decline in total phosphorus in the vessel is due to uptake by sessile organisms attached to walls of the vessel. Solid organic line, direct observations in water; dotted organic line, above plus decline in total (i.e. plus 100 minus total points of top line).

The center curve of Fig. 1 shows how inorganic phosphorus declines, mostly within the first day, to approach an equilibrium in which it is about equal to the organic fraction. The bottom curve, drawn through the analytical points, is organic ^{32}P in the water. To find the total organic production the decline as shown in the top curve should be added, to take in organic ^{32}P in sessile forms. The dotted line gives the revised values. From the lower curve (dotted line) the turnover time for organic phosphorus is calculated to be 19 hours, and for water, 27 hours. The turnover in a bottle experiment such as this one is likely to be more rapid than in an open lake, by reason of the glass surface available for bacterial growth. It was reported by Whipple (18) in 1901, and has been repeatedly verified since, that increase in surface is accompanied by an increase in the standing crop of bacteria in water.

This experiment may serve as well as any to illustrate the procedure for calculating turnover time. The required parameters are: concentration

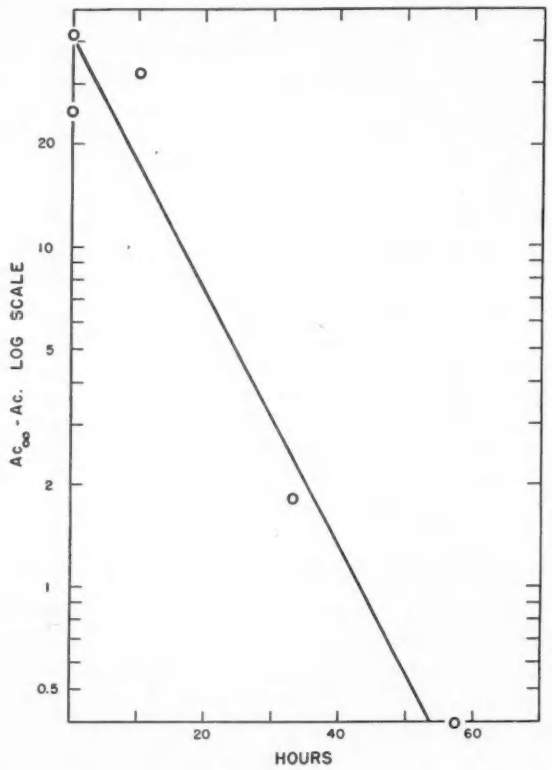


FIG. 2. Straight line plot for the conversion of inorganic ^{32}P to the organic form. Activity at equilibrium 41.5% minus activity at any time, t, is plotted logarithmically against time. The slope, 0.03876, is used when calculating the turnover times for water and organic phosphorus.

at zero time (which is zero) and final concentration when equilibrium has been reached. The latter is deduced in practice from inspection of the points making up the curve.

First the raw data are set down, then various values of Ac_∞ are tried. The correct value, which should be in the vicinity of the final observed value, gives the best straight line when $\log(Ac_\infty - Ac)$ is plotted against time (Fig. 2). In this example, it was 41.5. From the accepted $(Ac_\infty - Ac)$ line, the smoothed values are read off and the slope, k, is calculated, being:

$$\begin{aligned} \delta y/\delta x &= (\log 41.5 - \log 17.0)\ /10, \\ &= (1.6180 - 1.2304)\ /10, \\ &= 0.03876. \end{aligned}$$

From the equation

$$\begin{aligned} 1/T &= k/0.4343, \\ &= 0.03876/0.4343, \\ &= 0.0892, \end{aligned}$$

$$1/T = \lambda + \mu$$

where λ is the rate at which phosphorus is lost to the solid phase, and μ is the rate at which it is returned to the water.
Also

$$Ac_\infty = \lambda Ac_0/(\lambda + \mu)$$

where Ac_0 is the activity in the water at zero time.

$$\begin{aligned} \lambda &= [(\lambda + \mu)\ Ac_\infty]/Ac_0, \\ &= (0.0892 \times 41.5)\ /100, \\ &= 0.0370. \end{aligned}$$

$$\begin{aligned} \lambda + \mu &= 0.0892, \\ \lambda &= 0.0370, \\ \therefore\ \mu &= 0.0892 - 0.0370, \\ &= 0.0522. \end{aligned}$$

The turnover time, T_t, for inorganic phosphorus is $1/\lambda = 1/0.0370$,
$= 27$ hours.

For organic phosphorus, T_t is $1/\mu = 1/0.0522$
$= 19$ hours.

In words: a mass of inorganic phosphorus equal to the total mass present goes into and out of the organic state every 27 hours; a mass of organic phosphorus equal to its total in the system goes through the inorganic phase every 19 hours.

In a recent paper Rigler (14) reported that growing bacteria rapidly utilized inorganic phosphate from lake water, but when sterile water was stored there was no loss of phosphate from solution. He found very rapid turnover times

of inorganic phosphate between solution and organisms in several lakes in Ontario; these ranged from under 5 minutes to half an hour. His method was different from this author's in that his described equilibrium was between microorganisms and what passed through a molecular filter, i.e. was in true solution.

$$^{32}P \text{ in solution} \rightleftharpoons {}^{32}P \text{ in cells.}$$

In this paper the equilibrium described is

$$\text{inorganic } ^{32}P \text{ solution and microorganisms} \rightleftharpoons \text{organic } ^{32}P \text{ solution and microorganisms.}$$

The latter gives a turnover time which is an order of magnitude longer than Rigler reports for the former.

Artemia

One hundred *Artemia* were transferred from the culture aquarium to a battery jar containing 1 liter of filtered Halifax sea water. Radiophosphorus had been added to the water to give 720 counts/minute/ml. at zero time. Animals were removed from the battery jar at intervals, weighed on a torsion balance, and transferred to trays on which they were dried in an oven before they were tested for ^{32}P. Results are shown in Fig. 3, in which each point is the average for five animals. Each *Artemia* covered an area of approximately 0.5 cm.^{2} on the counting tray, and the average weight was only 9 mg. before drying so it was not necessary to correct for self-absorption of the radiation (7).

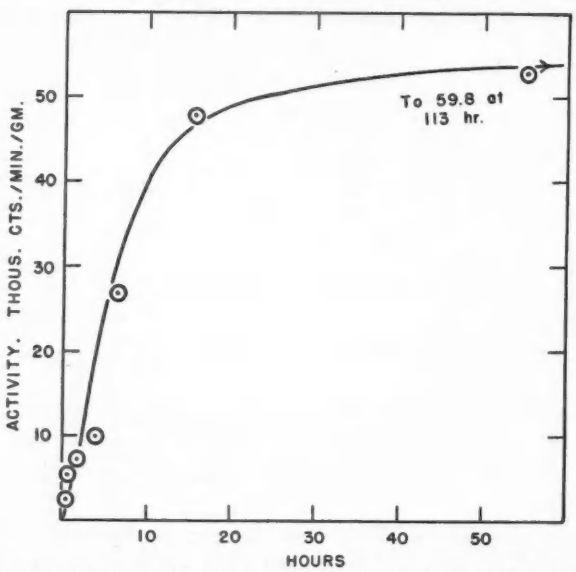


FIG. 3. Uptake of ^{32}P by the brine shrimp, *Artemia*. The experiment was conducted in sea water.

Fig. 3 shows that the uptake of radiophosphorus was very rapid at first and that it gradually tapered off to an equilibrium point. At equilibrium, each gram of *Artemia* contained 8.2% of the initial count of ^{32}P in the water. The results were plotted as a straight line as described in the previous section.

The turnover time for *Artemia* worked out to be 14 hours. As to the water, its ^{32}P is equilibrating with bacteria as well as with *Artemia* so that the latter are not to be equated with total phosphorus events in the aqueous phase.

Gammarus

Tests on marine *Gammarus* were made as described for *Artemia*. In two experiments the turnover times were 42.5 and 43.5 hours. The equilibration for one experiment is shown in Fig. 4. Some modification of the calculation is necessary because there was a negligible loss from the water, making λ very small and the turnover time for water infinitely long. Hence $\lambda + \mu$ is equal to μ and T is the same as T_t, being the turnover time for *Gammarus*.

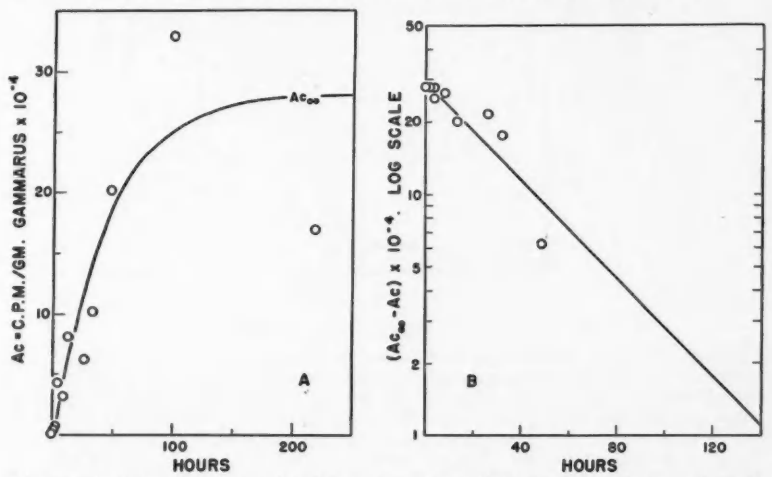


FIG. 4. Uptake of ^{32}P by marine *Gammarus*. At equilibrium the uptake was 28×10^4 c.p.m./g. wet weight *Gammarus* (A). Turnover time is calculated from the slope of the straight line plot (B).

Effect of Temperature

Gammarus, which had been acclimated to 3° to 5° C. in a refrigeration room, were washed in several solutions of filtered Halifax sea water before being placed in the experimental beakers. Pyrex beakers (800 ml.) were filled with 300 ml. of filtered sea water containing 1590 counts/minute/ml. of radiophosphorus. Eight animals were placed in each beaker. At intervals animals were removed from a beaker and analyzed for organic and inorganic radiophosphorus.

The experiment was repeated at room temperature (22°–25° C.) under the same conditions except that aeration was necessary to keep the animals alive at this temperature. The results are shown in Fig. 5. In order to bring

the two experiments into comparable units, the counts/minute/g. of *Gammarus* are divided by the counts/minute/ml. of sea water at zero time. This related the two experiments in which different concentrations of radiophosphorus were added to the sea water. The base line of Fig. 5 has been drawn logarithmically as observations were made at approximately logarithmically spaced intervals.

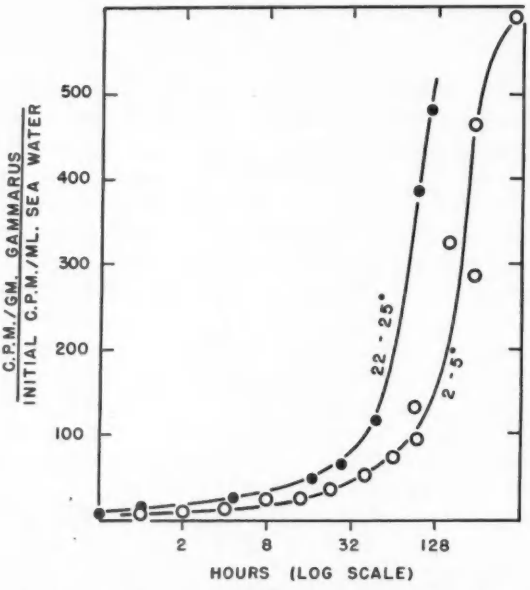


FIG. 5. Effect of temperature on uptake of radiophosphorus by *Gammarus*. To compare the two curves the c.p.m./g. *Gammarus* are divided by the initial c.p.m./ml. of the sea water used in each test. There was a time lag of a few hours before strong uptake began, of which the appearance is somewhat exaggerated by the logarithmic scale of the base line.

It will be noticed that neither experiment was carried far enough to extrapolate to the point of maximum uptake, hence is not usable to calculate turnover times. By inspection of Fig. 5, it may be observed that at 95 hours the animals at room temperature had taken up four times as much radiophosphorus as animals kept in the cold room. In both experiments relatively little radiophosphorus was taken up during the initial 32 hours. This point will be discussed later.

Fig. 6 shows the percentages of radiophosphorus found in organic combination during the experiments. For comparison, the percentage of phosphorus found in organic combination in non-treated controls is indicated on the graph. The first observations are within the normal range ($63.7 \pm 4.9\%$) for organic phosphorus in the animals. The organic phosphorus rises to a peak of 85% and then falls back to the normal range. Apparently the radiophosphorus equilibrates with labile compounds in the body fluid in a matter of minutes.

The turnover rate of more stable phosphorus-containing organic compounds such as proteins and nucleic acids is much slower. As radiophosphorus becomes incorporated into the stable compounds the percentage of organic ^{32}P in the animals reaches a peak. At this stage ^{32}P is no longer serviceable as a tracer, being in complete equilibrium with the ordinary phosphorus throughout the animal. It is surprising that at room temperature, 60% of the ^{32}P in the animals has been converted to the organic form only ½ hour after its addition to the water in the inorganic form.

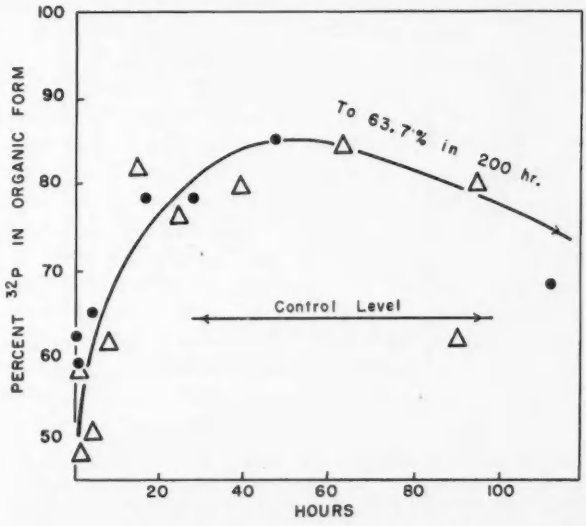


FIG. 6. Percentage of ^{32}P which is in the organic form in *Gammarus*, following immersion in active sea water. As soon as any of the phosphorus is found the organic fraction approaches the normal value. An above-normal peak occurs at 2–3 days, followed by a gradual decline to reach the normal value again after about a week. The horizontal line marked "control" is the analysis for untreated specimens, whose organic phosphorus is 63.7 ±4.9%. Dots are a test at 22–25°; triangles are a test at 2–5° C.

Effect of Antibiotic

The first experiment described showed that bacteria in the water make over a large part of inorganic ^{32}P to the organic form in a few hours. In tests with invertebrates there were always bacteria in the water. It was of interest to determine whether bacteria are necessary intermediates in the invertebrate effect, and *Gammarus* experiments were set up with and without pretreatment by the antibiotic terramycin. The flasks used had 300 ml. of autoclaved sea water to which ^{32}P was added, and were stoppered with absorbent cotton.

The minimum was one treatment with terramycin at a strength of 100 mg. % (see Methods). Here the initial ^{32}P in water was 1822 c.p.m. per ml.

The intermediate was two antibiotic treatments as above, the initial ^{32}P being 2029 counts.

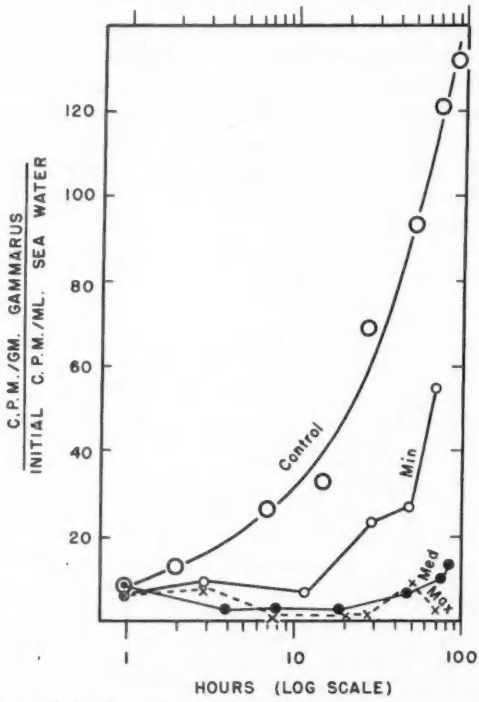


FIG. 7. Uptake of ^{32}P by *Gammarus* after antibiotic treatment compared with control. Three levels of pretreatment with terramycin are shown as minimal, medium, and maximal. The antibiotic caused a great reduction of bacteria in the water, which is reflected in a diminished uptake of ^{32}P by the organisms. Evidently the intermediary activity of bacteria is necessary to permit inorganic phosphorus to be used.

The maximum was three treatments at 24-hour intervals with terramycin at 250 mg.% strength. This is approaching the level of toxicity. Sea water started with 1264 c.p.m.

Results are shown in Fig. 7. In all three terramycin experiments the uptake was considerably less than that of the control. The only point where a large amount of radiophosphorus was taken up was at 70 hours in the first or minimal terramycin experiment. This flask contained over 100,000 bacteria per ml. of sea water, considerably more than in any of the other flasks. The control ran to about 1.25×10^6 colonies of bacteria per ml. sea water. In the weakest and medium terramycin tests there were initially about 200 colonies, rising to 5000 during the test (except for the one final high value mentioned). Following the maximal antibiotic pretreatment the colonies started at 5 per ml. and went up to nearly 500 by the end.

Discussion

The experimental results may be summarized as follows: Radiophosphorus equilibrates very quickly between organic and inorganic fractions in lake waters because of the uptake by microorganisms. It also equilibrates very

quickly between *Artemia* and water, and more slowly between *Gammarus* and water. In *Gammarus* there is a lag period in which uptake is slow. Sterile *Gammarus* take up hardly any radiophosphorus.

From the results it is evident that *Gammarus* does not take up appreciable quantities of phosphorus by direct absorption through the body wall, intestine, or gills. This is in agreement with the findings of Bond (1) and Krogh (10). Bond concluded that, in general, marine invertebrates are impermeable to water, salts, and organic solutes. From experiments on *Artemia* in which he used heavy water as an indicator in the medium, Krogh found only a slight exchange between the water of the blood and that outside. Two alternative methods remain by which *Gammarus* may obtain its phosphorus, (*a*) in organic combination by ingestion of the dissolved organic material in the water, or (*b*) by digestion of particulate matter.

The first method indicated above was first postulated by Pütter (13). Krogh (9) reviewed the subject and concluded that no multicellular animals take up dissolved organic substances from natural water in any significant amount, although certain Protozoa may do so. Stuart, McPherson, and Cooper (16) raised bacteriologically sterile *Moina macrocopa* and found them unable to subsist on dissolved organic material. Particulate food in the form of living bacteria (killed bacteria did not support successive broods) allowed the cladoceran *Moina* to be cultured indefinitely. Gellis and Clark (3) found that *Daphnia magna* could derive nourishment from colloidal organic matter, but this substance played an insignificant role as food for the animals.

In the present investigation there was no organic radiophosphorus except that produced by bacteria after the experiment began. The terramycin experiments which have been described make it appear probable that the animals obtain radiophosphorus as particulate matter in the bodies of the bacteria. The availability of bacteria as food for crustaceans depends upon the numbers and species of bacteria in the medium, as well as the length of time the animals are allowed to feed on them. The last point may be used to explain the initial, relatively slow uptake of radiophosphorus by the controls. Fig. 1 shows that bacteria require nearly 40 hours before they begin to equilibrate with the inorganic radiophosphorus in the solution. About the same length of time is required before *Gammarus* begin to take up the radiophosphorus more quickly. To state this differently, there are more bacteria containing radiophosphorus after about 40 hours, so if the animals are feeding on the bacteria at a constant rate they will take up more radiophosphorus in the bodies of the bacteria after 40 hours.

Stuart *et al.* (16) showed that certain species of bacteria are better as food for *Moina* than others. Hutchinson (6), from a review of the literature, suggests that "Selective feeding is to be expected, even in planktonic Calanoida . . . objects that are too large for the small species being readily ingested by the large". *Artemia* feed for the most part on Protozoa, unicellular algae, bacteria, and finely divided detritus (11). Their filtration mechanism is so efficient that particles 0.25 μ in diameter, and probably less, are secured in large numbers.

It has been shown by ZoBell (20) that the numbers of bacteria which grow in containers vary directly with the relative surface area of the container. This means that more bacteria grow per unit volume in a laboratory container than in a lake, a fact which must be taken into consideration when interpreting laboratory experiments. Under natural conditions the aquatic invertebrates also have access to unicellular algae, diatoms, and other small organisms.

References

1. Bond, R. M. A contribution to the study of the natural food cycle in aquatic environments. Bull. Bingham Oceanog. Collection, **4**, 1–89 (1933).
2. Coffin, C. C., Hayes, F. R., Jodrey, L. H., and Whiteway, S. G. Exchange of materials in a lake as studied by the addition of radioactive phosphorus. Can. J. Research, D, **27**, 207–222 (1949).
3. Gellis, S. S. and Clark, G. L. Organic matter in dissolved and in colloidal form as food for *Daphnia magna*. Physiol. Zool. **8**, 127–137 (1935).
4. Hayes, F. R. and Jodrey, L. H. Utilization of phosphorus in trout as studied by injection of radioactive phosphorus. Physiol. Zool. **25**, 134–144 (1952).
5. Hayes, F. R., McCarter, J. A., Cameron, M. L., and Livingstone, D. A. On the kinetics of phosphorus exchange in lakes. J. Ecol. **40**, 202–216 (1952).
6. Hutchinson, G. E. Copepodology for the ornithologist. Ecol. **32**, 571–577 (1951).
7. Kamen, M. D. Radioactive tracers in biology. Academic Press, Inc., New York. 1948.
8. King, E. J. The colorimetric determination of phosphorus. Biochem. J. **26**, 292 (1932).
9. Krogh, A. Dissolved substances as food of aquatic organisms. Biol. Revs. **6**, 412–442 (1931).
10. Krogh, A. Osmotic regulation in aquatic animals. Cambridge Univ. Press, London. 1939.
11. Lochhead, J. H. *Artemia* the "brine shrimp". Turtox News, **19**, 41–43 (1941).
12. McCarter, J. A. and Steljes, E. L. Methods for the determination of the distribution of radioactive phosphorus among the phosphorus containing constituents of tissues. Can. J. Research, E, **26**, 333–346 (1948).
13. Pütter, A. Die Ernährung der Wassertiere und der Stoffhaushalt der Gewässer. Jena, 1909.
14. Rigler, F. H. A tracer study of the phosphorus cycle in lake water. Ecol. **37**, 550–562 (1956).
15. Spencer, C. P. On the use of antibiotics for isolating bacteria free cultures of marine phytoplankton organisms. J. Mar. Biol. Assoc. U.K. **31**, 97–106 (1952).
16. Stuart, C. A., McPherson, M., and Cooper, H. J. Studies on the bacteriologically sterile *Moina macrocopa* and their food requirements. Physiol. Zool. **4**, 87–100 (1931).
17. Welch, P. A. Limnology. 2nd ed. McGraw-Hill Book Company, Inc. New York, N.Y. 1952.
18. Whipple, G. C. Changes that take place in the bacterial contents of waters during transportation. Tech. Quart. **14**, 21–29 (1901).
19. Willard, H. H. and Diehl, H. Advanced quantitative analysis. D. Van Nostrand Company, Inc., New York. 1943.
20. ZoBell, C. E. and Anderson, D. Q. Observations on the multiplication of bacteria in different volumes of stored sea water and the influence of oxygen tension and solid surfaces. Biol. Bull. **71**, 324–342 (1936).

THE AVIAN BLOOD PARASITE BABESIA MOSHKOVSKII (SCHURENKOVA, 1938), WITH A RECORD FROM CORVUS SPLENDENS VIEILLOT IN PAKISTAN[1]

MARSHALL LAIRD[2] AND FAIYAZ A. LARI[3]

Abstract

A description is given of a haematozoan from an Indian house crow, *Corvus splendens* Vieillot, from Karachi, Pakistan. This organism is identified as *Babesia moshkovskii* (Schurenkova, 1938), following a review of the literature. Other hosts for *B. moshkovskii* include the domestic fowl, which also harbors the better known babesioid *Aegyptianella pullorum* Carpano, and eagles, herons, kestrels, and owls in Tadjikistan (U.S.S.R.), Indo-China, and Egypt.

This parasite was the only babesioid encountered during a random survey of avian haematozoa in Pakistan. It was discovered in a Giemsa-stained heart-blood film taken from an Indian house crow, *Corvus splendens* Vieillot, at Karachi, on October 7, 1954. A complete list of the various birds examined will appear in a paper submitted for publication elsewhere (20).

Descriptive Account

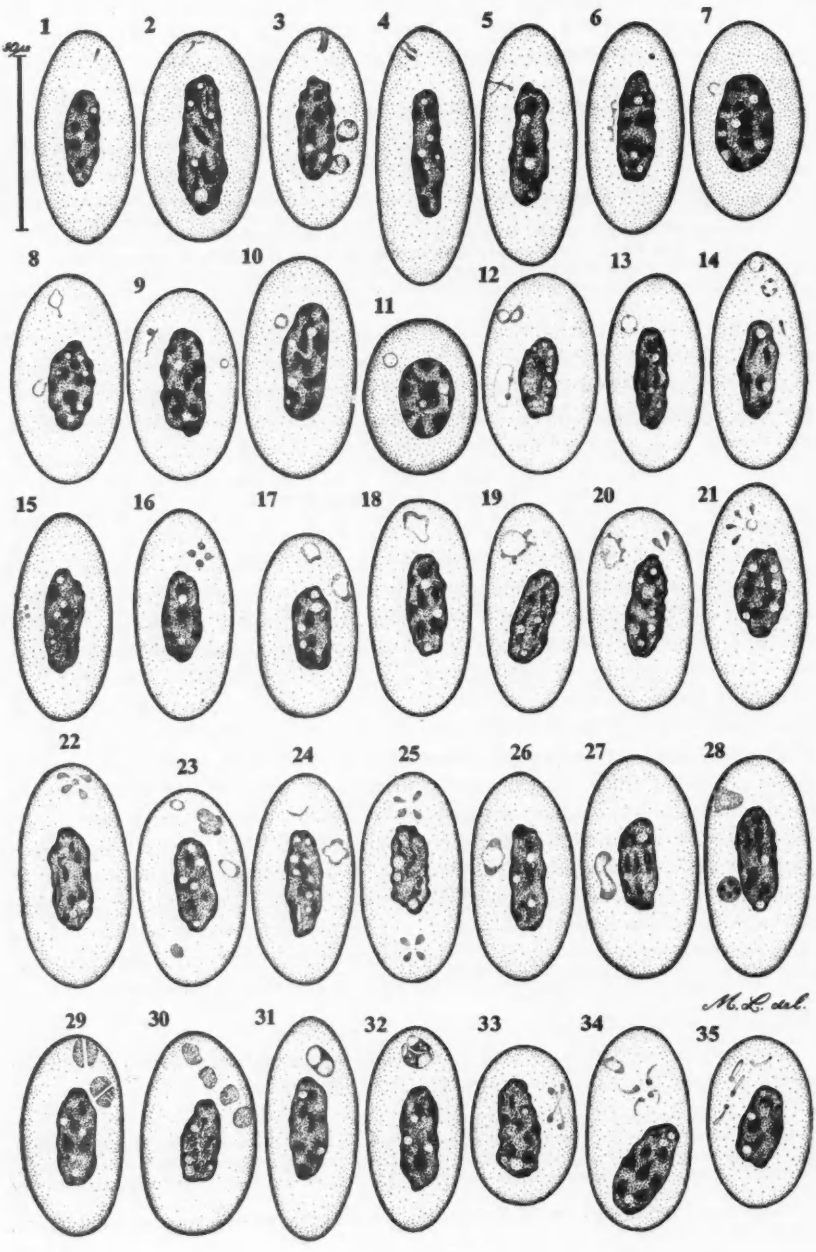
General

Six per cent of the red cells in the film contain parasites. Although most of the infected cells are mature erythrocytes, both trophic and dividing parasites may occur in polychromatophil erythroblasts (Figs. 11, 33) and normoblasts (Fig. 2) as well. All stages of the organism are devoid of pigment, and are most commonly located towards one of the poles of the host cell. The fairly high parasitemia, an abundance of dividing forms, and the frequent occurrence of two (Figs. 3, 9, etc.) or even three (Fig. 14) different life history stages in a single erythrocyte indicate that the infection was probably in its acute phase. Parasitized cells are neither hypertrophied nor distorted, the range in size of the cells illustrated in the figures being in accordance with that of parasite-free ones in the same preparation. The host cell nucleus is sometimes displaced (Figs. 19, 33, 34). The considerable size range of uninfected red cells and the occurrence of numerous normoblasts and erythroblasts, some of the latter in mitosis, are indicative of anemia; but as we have no data concerning the course of the infection it cannot be stated whether this condition is attributable to the parasite or to some other cause.

Anaplasmoid Bodies

These consist of minute dots of red-staining material, and lack any indication of cytoplasm (Fig. 6, top). Diameter, 0.2 to 0.6 μ.

[1] Manuscript received September 12, 1957.
Contribution from the Institute of Parasitology, McGill University, Macdonald College, Macdonald College P.O., Que., Canada, and the Malaria Institute of Pakistan, Karachi, Pakistan. Preliminary work was carried out in the Department of Parasitology, University of Malaya, Singapore.
[2] Institute of Parasitology, McGill University. Formerly of the Department of Parasitology, University of Malaya.
[3] Malaria Institute of Pakistan (Karachi Branch).


20μ
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
M.L. del.
29
30
31
32
33
34
35

Elongate Forms

Some of these (Figs. 1, 14) exhibit a terminal dot of chromatin and a slender, usually straight, cytoplasmic tail, their dimensions ranging from 0.7 to 0.9 μ by 0.1 μ. Others often assume a uniformly reddish stain (Figs. 6, 24), may be of irregular outline (Figs. 6, 9) or crescentic (Fig. 24, top), and range up to 2.5 μ in length.

Ring Forms

The smallest ones (Figs. 9–11) usually stain uniformly red, and are of a diameter of 0.4 to 0.9 μ. Rather larger ones, which show differentiation between cytoplasm and chromatin (Fig. 14, top), greatly resemble signet ring trophozoites of *Plasmodium*. The central vacuole assumes a lighter stain than the surrounding host cell cytoplasm, and may appear whitish and somewhat refractile. The oldest rings are often of oval to irregular outline, and measure from 1.2 by 0.9 μ to 2.1 by 1.4 μ (Figs. 17, 18, 23).

Large, Solid Forms

These may be round, oval, or of irregular outline (Figs. 3, right; 23, bottom; 28). They range from 0.9 to 5.3 μ in diameter, averaging 1.5 μ. Chromatin appears to be thinly diffused throughout the cytoplasm, the color assumed with Giemsa ranging through various shades of red to violet.

Binary Fission

All stages have the faculty of dividing by simple binary fission. Broadly V-shaped elongate forms having a central chromatin mass at the junction of two blue or reddish-staining arms (Fig. 2) are quite common. In the case of pairs like that illustrated in Fig. 3 (top), the two straight or slightly curved components lie closely parallel to one another, usually near or in contact with the cell membrane, and assume a uniformly reddish stain. Each member of such a pair may itself divide into two by transverse binary fission (Fig. 4). Horseshoe-shaped elongate parasites having a knob at either end (Figs. 7, 8, bottom) are perhaps in process of division.

Rings having a knobbed projection (Fig. 8, top) are occasionally seen, but are of unknown significance. Those undergoing simple binary fission have the appearance of a figure-of-eight just before cytoplasmic cleavage takes place (Fig. 12, top), and it is common to find two closely associated rings of similar size in the one erythrocyte. Dividing rings having a bipolarity due to the presence of a large mass of chromatin at either end are illustrated in Figs. 26, 27, and 34.

The large, solid forms of the organism exhibit both longitudinal and transverse binary fission, the line of cleavage being quite straight (Fig. 29).

A very few oval parasites, rich in cytoplasm and having an intensely staining nucleus in process of division, have been seen (Fig. 31). These are of relatively large size, ranging from 2.0 by 1.5 μ to 5.8 by 3.3 μ. They form two pyriform daughter individuals having a central nucleus, which stains bright red, and abundant sky-blue cytoplasm (Fig. 32). The subequal

division products resemble those of the larger species of *Babesia* such as *B. bigemina* (Smith and Kilborne), while their *tête-bêche* arrangement bears comparison with that of *Haemogregarina bigemina* Laveran and Mesnil (19).

Schizogony

Cruciform (Figs. 5, 33) and fan-shaped (Figs. 19, 20) schizonts are not uncommon, although less plentiful than the stages thus far considered. They are of various sizes, small cruciform ones often being uniformly red staining and forming four tiny anaplasmoid merozoites lacking any indication of cytoplasm (Fig. 15). Such division forms are very like those of the babesioids for which Neitz and Jansen (25) proposed the new family name of Gonderidae (see p. 788). It is possible that merozoites of this first type may also result from the division of schizonts of the kind illustrated in Figs. 12–14, in which from two to four chromatin beads appear strung on a thread-like circlet of cytoplasm. Sometimes four small merozoites, still closely associated in box formation, each exhibit a tiny, outwardly-directed tail of cytoplasm (Fig. 16).

Somewhat larger schizonts give rise to merozoites of a second type, pyriform and uniformly red-staining structures the dimensions of which range from 0.5 by 0.2 μ to 0.7 by 0.5 μ. Groups of four such merozoites resulting from the division of cruciform schizonts are initially disposed in box formation with their tapered ends pointing in towards the common center. In some cases there is no trace of any residual material (Fig. 25), while in others there is a central reddish-staining mass (Fig. 22), probably consisting of cytoplasm with some diffused chromatin. Similar merozoites may be produced in another fashion, from large rings having a relatively heavy lateral mass of chromatin (Fig. 18) which gives rise to four subequal buds resembling the teeth of a ratchet (Figs. 19, 20). The cytoplasm of such schizonts becomes isolated as a ring-shaped residual body at merozoite formation (Fig. 21). Once only, two pyriform merozoites of the second type were noted lying close together in a red cell (Fig. 20, right). While these may perhaps have been formed by binary fission, it is equally likely that they were produced by schizogony with the separation of four merozoites, their two associates having already made their way out of the host cell.

Certain large, cruciform schizonts have a proportionately greater amount of cytoplasm (Fig. 33), their chromatin being concentrated in knobs at the points of the division figure. A third type of merozoite is formed from these, having a rounded chromatin head 0.2 to 0.3 μ in diameter, and a straight or curved cytoplasmic tail 0.7 to 1.1 μ long (Figs. 34, 35). Despite the fact that the chromatin of such schizonts is always peripheral, the heads of the four separated merozoites are usually inwardly directed (Fig. 34). Perhaps this is due to a rapid post-division reorientation, for the curved tails of the merozoites suggest active motility as pointed out by Mohamed (23). Before quitting the host cell the merozoites may move about within it to some extent (Fig. 35), and at this stage their cytoplasm frequently appears to be an elongate loop rather than a solid tail.

The chromatin of parasites of the solid, red-staining type, may become concentrated peripherally (Fig. 23, top right), after which a central vacuole becomes evident (Fig. 24, right) and four merozoites are formed. Other schizonts developed from trophozoites of this type have four (sometimes as many as six) internally located nuclei (Fig. 28, bottom). These bear comparison with the equivalent stages of *Aegyptianella*, and with schizonts of the type described from Iranian *Babesia* (= *Luhsia*) *bigemina* by Delpy (11).

Finally, it must be noted that although no endoleucocytic stages were seen, some lymphocytes and monocytes held a number of small (0.5 to 1.8 μ) red-staining inclusions interpreted as phagocytosed chromatin probably of parasite origin.

Relationship of the Various Stages to One Another

This cannot be assessed with any finality from the very limited material available. It would seem that anaplasmoid merozoites of the first type reinvade red cells to initiate a further cycle of development, but whether elongate parasites like the ones illustrated in Figs. 1 and 9 are derived from these or from the second or third types of merozoites is a matter for conjecture. Furthermore, it is by no means clear whether the various manifestations of binary fission and schizogony described are indicative of regularly repeated stages in a definite sequence of development, or whether they are evidence for Rousselot's (29) contention that babesioids in general have an innate capacity to divide in these different ways throughout their cycle in the vertebrate host. The great difference in size between all the division products of schizonts of the cruciform types and the much larger pyriform bodies illustrated in Fig. 32 is in accord with that between merozoites and gametocytes or gametes in other Sporozoa, but confirmation of this and elucidation of the nature of the large, red-staining stages must await further studies.

Systematic Position

The lack of pigment and the method of division in red cells refer this organism to the sporozoan group commonly known as the piroplasms, from the superseded generic name of *Piroplasma* Patton, 1895. This name, which formerly enjoyed wide currency, is now generally recognized as a synonym of *Babesia* Starcovici, 1893 (35). The status and interrelationships of the group, herein referred to as the babesioids, are still incompletely understood, various authors having sought to assign them to the Coccidia or Haemosporidia, or even to the Sarcodina. For present purposes it is considered best to follow Poisson (27) in regarding them as Sporozoa of uncertain standing. Poisson established the new superfamily Babesioidea for their reception, the summarized diagnosis being as follows:

> Parasites of red (and in some cases white) cells of vertebrates, especially mammals. Simple, pigment-free structures of small size, rounded, pyriform, or bacilloid. Asexual reproduction by

binary or multiple fission or by budding.* Sexual multiplication also takes place, although gametocytes have only been identified with certainty in one of the families. Ticks are the sole known vectors.

Three families, the Anaplasmidae, Babesiidae, and Theileriidae, were included in the Babesioidea by Poisson (27), and a fourth, the Dactylosomidae, has since been added by Jakowska and Nigrelli (17). The Anaplasmidae, tiny *Coccus*-like organisms devoid of cytoplasm, occur in mammalian red cells; the Babesiidae, which multiply asexually in erythrocytes by binary fission or schizogony, parasitize mammals, birds, and reptiles; the Theileriidae are mammalian parasites which undergo schizogony in white cells, their gametocytes occurring in red cells as small round, oval, or bacilloid bodies; and the Dactylosomidae are parasites of reptiles, amphibians, and fish, producing schizonts which form 4–16 merozoites in circulating red blood cells. According to Jakowska and Nigrelli (17, 18) the gametocytes of members of the latter family are also developed in the erythrocytes. If this is indeed so, it would seem to be the only major criterion distinguishing the Dactylosomidae from the Babesiidae, and further research might invalidate this distinction, most of what has been written concerning gametogony in the latter family being of a speculative nature; for, although asexual multiplication in the Babesiidae usually results in the production of only two or four merozoites, there are good grounds for following Poisson in assigning the avian and reptilian babesioids of the genus *Aegyptianella* Carpano, which form much larger numbers of endoerythrocytic merozoites, to this family.

Very little is known of the cycle of any of these organisms in the invertebrate host, and complete studies of babesioid life histories will no doubt indicate the need for a revision of the scheme of classification adopted herein. However, as matters stand, Poisson's approach has the merit of grouping these parasites into a natural assemblage within which interrelationships can be established without prejudice to questions of classification at a higher level.

Anaplasmoid stages like those described herein from the *Corvus* parasite frequently form part of the cycle of representatives of both the Babesiidae and the Theileriidae. Members of the latter family are generally smaller and less rich in cytoplasm than those of the former one, but the two families cannot always be separated from one another on the grounds of the morphology of forms present in circulating red blood cells. There is particular difficulty in regard to small babesiids of the type of *Babesia* (*Nuttallia*) *equi* (Laveran) and certain theileriids for which Neitz and Jansen (25) have recently proposed the new family name of Gonderidae, as small cruciform schizonts are formed in red cells in both cases. Nevertheless, although the anaplasmoid cross forms of our parasite bring these theileriids to mind, the lack of endoleucocytic schizonts and the occurrence of trophozoites and schizonts having considerable cytoplasm, and of rare, pyriform bodies of relatively large size (Fig. 32), are

* We prefer to express this as "Asexual reproduction by binary fission or schizogony", following Wenyon (35), who pointed out that the process of budding into two or four commonly seen in babesioids does not differ fundamentally from normal sporozoan schizogony.

all compatible with the family Babesiidae. Some authors, Wenyon (35), Thomson and Hall (32), and Neitz (24) for example, admit only one genus, *Babesia* Starcovici, to this family. Others, including Neveu-Lemaire (26), Sergent *et al.* (31), and Poisson (27), differentiate two or more genera, to several of which the babesioids previously described from birds have been assigned.

Specific Identity

The earliest record of avian babesioids is that of Lingard and Jennings (21), who referred to the occurrence of "piroplasms" in domestic fowls in India. Three years later Balfour (2) published a preliminary note on what he believed to be "a true piroplasm" occurring in association with spirochetes in the blood of the same host in the Sudan. Dschunkowsky and Luhs (12) discovered endoerythrocytic bodies, resembling those described by Balfour, in Transcaucasian fowl, but felt that these were perhaps mere fragments of chromatic material of similar nature to Kurloff's bodies. In regard to this suggestion it should be noted that Porter (28) described erythroblastic nuclear remnants superficially resembling *Anaplasma* from a variety of experimental animals, including birds, and that *Anaplasma*-like bodies were reported by Wasielewski (34) from the little owl, *Athene noctua*.

Balfour (3), in describing *Spirochaeta granulosa penetrans* from Sudanese fowl, dealt at some length with the subject of avian spirochetosis. He concluded that the associated bodies he had previously considered to be piroplasms actually represented an asexual phase in the life history of *S. granulosa penetrans*, claiming that granules discharged by the spirochetes entered erythrocytes, developed further therein, and in due course underwent schizogony. A few years later Balfour (4) was inclined to revert to his earlier opinion that two distinct organisms were involved, other authors having by then published accounts of fowl spirochetosis unassociated with granular erythrocytic inclusions. The latter remained generally known as "Balfour's bodies" for several years. Franchini (14) drew attention to the occurrence of similar bodies without an associated spirochetosis in an Italian bird, *Hypoleis hypoleis*, and Wenyon (35), who reviewed the literature on avian spirochetosis and concluded that all the spirochetes until then recorded from fowl, geese, and ducks are properly referable to *Treponema anserinum* (Sakharoff), considered Franchini's discovery as support for the hypothesis that "Balfour's bodies" belonged to an unknown group of parasites. It was left for Carpano (7) to show that these bodies are in fact closely related to *Babesia*. Their distinctive schizogony separating them from previously known babesioids, he established a new genus with *Aegyptianella pullorum* as its type. Carpano (8) extended the host range of his species to geese, and Farid (13) recorded it from turkeys and guinea fowl in Egypt. Bedford and Coles (6) established that the vector, like those of other babesioids, is a tick. The species concerned, *Argas persicus*, also transmits the fowl spirochete. There are now records of *A. pullorum* from domestic birds in various tropical and

subtropical countries, from feral quail, and from a number of laboratory hosts including pigeons and canaries. A second species, *Aegyptianella carpani* Battelli (5), has been described from a snake.

The following brief description of *Aegyptianella pullorum*, the only avian babesioid to have attained general recognition thus far, is compounded from various authorities. The earliest stages present in red cells are oval or rounded granules less than 1 μ in diameter, staining red with Giemsa, and usually located towards the cell membrane. A tiny amount of cytoplasm is sometimes detectable. Developing parasites, which may be round, oval, pyriform, or of irregular outline, range from 1 to 2 μ in diameter. They are differentiated into cytoplasm and chromatin, and sometimes have a central vacuole. Schizonts are of various shapes and measure some 3 to 4 μ by 2 to 2.5 μ. There may be from one to four central, pale-staining chromatic bodies, and deeper-staining nuclei become disposed peripherally. From 6 to 20 (frequently 12) minute merozoites are formed. Finally, large round or oval parasites, assuming an intense stain and very rich in chromatin, are quite common. Carpano considered that these are perhaps gametocytes.

Poisson (27) included *Aegyptianella* in the Babesiidae on the grounds of the absence of pigment, the morphology of some of the developmental stages, and the method of division in red cells. The high average merozoite number characterizing this genus serves to distinguish it from *Babesia*, the same criterion having been used by Jakowska and Nigrelli (18) to differentiate *Dactylosoma* Labbé from their new genus *Babesiosoma*.

While there are close resemblances between the trophic stages of *Aegyptianella* and the babesioid of *Corvus splendens*, the latter differs from the former but agrees with numerous other members of the Babesiidae in exhibiting binary fission and schizogony with the characteristic production of four merozoites from fan-shaped or cruciform division figures.

Schurenkova (30) established the genus *Sogdianella* for a babesioid of an eagle (*Gypaëtus barbatus*) in the Russian province of Tadjikistan. This organism, *S. moshkovskii*, resembles ours very closely indeed. It commonly produces four merozoites from schizonts of the type illustrated in Figs. 19 and 20 herein, each merozoite having a tiny, outwardly directed cytoplasmic tail and the four being grouped about a central residual body (cf. Schurenkova's Fig. 7). Large homogeneous bodies like those formed by the Pakistan parasite were viewed as gametocytes by Schurenkova, who considered reddish-violet inclusions of circulating leucocytes and of the Kupffner's cells of the liver as evidence of schizogony in white cells.

Henry (15) described (but failed to name) a small fowl babesioid differing from *Aegyptianella pullorum*. From his description and figures, this too is closely comparable with the species under consideration. Henry's parasite ranges from 0.5 to 2.5 μ in diameter, occurring as *Anaplasma*-like granules staining red with May-Grunwald-Giemsa, as small rings and as elongate bodies having a terminal dot of chromatin and a thin tail of cytoplasm (compare Figs. 1 and 14 herein with Henry's 2nd and 17th figures). Both binary

fission and schizogony take place, the schizonts having their nuclei either strung on a thin cytoplasmic ring (cf. Figs. 13 and 14 herein) or at the angles of triangular or lozenge-shaped figures. In the latter case the merozoites either separate with no indication of residual material (cf. Henry's 20th figure and Fig. 15 herein), or else they are grouped about a central residual body as is often the case with the *Corvus* parasite. Schizonts usually have four nuclei, although some have as many as six. Some of the numerous illustrations published by Balfour in the course of his studies of fowl haematozoa show small stages including the cross arrangement of four anaplasmoid granules. These differ so much from typical *Aegyptianella* schizonts that one must not overlook the possibility that he was dealing with mixed infections of *A. pullorum* and a smaller babesioid comparable with that described by Henry.

The Indo-Chinese heron *Ardea cinerea* var. *rectirostris* is host to an organism described by Toumanoff (33) as *Babesia* (*Nicollia*) *ardeae*. This polymorphic red cell parasite occurs as tiny rings and as pyriform or comma-, V-, horseshoe-, or irregularly-shaped bodies. Some examples stain a uniform red with Borel blue, while others exhibit differentiation between cytoplasm and chromatin. Binary fission takes place, and Toumanoff illustrated reniform and circular binucleate and tetranucleate schizonts agreeing with examples in our preparation (cf. his Figs. 10 and 13; Figs. 12 and 13 herein). Four oval or pyriform merozoites commonly appear disposed fan-wise or in the form of a cross. Toumanoff's figures show no indication of a residual body, but the nucleated ends of the merozoites face inwards as in the present case. Following Wenyon (35), Toumanoff recognized but one genus, *Babesia*, in the family Babesiidae. However, he employed the subgeneric designation of *Nicollia* Nuttall, a name originally proposed for a babesioid supposed to have two unequal nuclei throughout the trophic phase, and forming four merozoites from fan-shaped or cruciform schizonts. Most recent authors regard *Nicollia* merely as a synonym of *Babesia*. Even should it prove that the binuclearity of *Nicollia* is a constant character of taxonomic significance, *B. ardeae* would not belong there. Toumanoff himself stated that uninucleate as well as binucleate trophozoites occurred in his material, and, as Mohamed (23) has pointed out, there is thus no reason for assuming that the latter were anything other than early division stages.

Mohamed (23) proposed the name of *Nuttallia shortti* for a babesioid of the Egyptian kestrel, *Falco tinnunculus* var. *rupicolaeformis*. He gave a very detailed description of this organism, which he succeeded in subinoculating to *Falco n. naumanni*, and noted the occurrence of a morphologically similar parasite in nestling barn owls, *Tyto a. alba*. Mohamed drew attention to the great diversity in shape, structure, and size exhibited by *N. shortti*. He grouped the various stages under a number of headings broadly corresponding with those employed herein, his parasite having an anaplasmoid stage (0.5 to 1.0 μ), elongate forms of various shapes and of a thickness of 0.25 μ or less to 0.75 μ, signet ring trophozoites (0.5 to 2.0 μ), and roughly ovoid forms attaining a maximum diameter of 4.0 μ. Elongate and ring forms

undergo binary fission, and fan-shaped and cruciform schizonts give rise to four merozoites the nuclei of which are generally central despite the fact that those of the schizonts are peripheral. Mohamed considered that his parasite differed specifically from *Babesia ardeae* on zoogeographical grounds and because elongate forms of the latter sometimes (although only rarely, according to Toumanoff) reach a length of almost 6 μ, and held it to be distinct from *Sogdianella moshkovskii* in lacking any indication of endoleucocytic stages and because of the higher size limit of the latter species (8 μ, by comparison with 4 μ). He overlooked the babesioid described by Henry (15). The only respects in which our parasite is at variance with *N. shortti* are the rather greater diameter attained by the largest stages (5.8 as opposed to 4.0 μ) and its occasional formation of as many as six merozoites from one schizont. Otherwise agreement is complete, and extends to the types of host cells infected, the location of the parasites in these cells, and the capacity to displace the host cell nucleus.

There are several further records of avian babesioids in the literature, but data concerning schizogony are lacking in all cases. Two investigators familiar with *Aegyptianella pullorum* have noted small ring forms (0.5 to 1.5 μ, av. 1.0 μ diameter) of a different babesioid in the red cells of fowl. Coles (9) discovered this organism in material from the U.S.A. (New York and Philadelphia) and South Africa, and Abdussalam (1) found it in association with *A. pullorum* in the Punjab. MacNeil and Hinshaw (22) reported a morphologically similar organism from American turkey poults. The ring forms of Coles' parasite are sometimes uniformly red staining, and the occurrence of bipolar examples (very like that illustrated in Fig. 34 herein) and numerous closely associated pairs suggests that binary fission takes place. Henry (15) remarked upon the close agreement between this parasite and equivalent stages of that which he described in greater detail from the same host. Coles (10) recorded what he believed to be an *Aegyptianella* in spirochete-infected penguins (*Spheniscus demersus*) on Dassen Island, off the South African coast, and Rousselot (29) accorded the name of *Piroplasma avium* to small (0.5 to 2.0 μ) signet ring parasites from pheasant in Iran, and listed several domestic and feral hosts for an *Aegyptianella*-like organism which he identified as *A. pullorum*. No conclusions as to the identity of any of these insufficiently described babesioids, or of Franchini's parasite of *Hypoleis hypoleis*, can be drawn at this juncture.

However, there is a very considerable measure of agreement between the fowl babesioid described by Henry (15), *Babesia ardeae* Toumanoff, *Nuttallia shortti* Mohamed, the present parasite of *Corvus splendens*, and the erythrocytic stages of *Sogdianella moshkovskii*.

Mohamed attached a considerable measure of importance to the great distance separating Egypt from Indo-China in arguing the specific distinctness of *N. shortti* and *B. ardeae*. This argument loses force in the face of the occurrence of morphologically similar babesioids in intermediate localities in Tadjikistan and Pakistan. In any case, various other sporozoan parasites

of birds, notably species of *Plasmodium*, are known to have a much wider distribution than this. Avian blood Sporozoa are frequently found in numerous hosts only distantly related to one another, their distribution being correlated with that of suitable vectors rather than with that of particular species of birds. In this connection, Schurenkova (30) considered the possibility that the young eagle from which her parasite was recorded might have acquired its infection from a tick off a mammal brought to the nest by a parent bird, although she felt that infection by a specific avian tick was more likely; and Mohamed (23) pointed out that not only do eagles, kestrels, and owls prey upon small mammals, but that even herons may occasionally do so. A rather different elaboration of this ecological theme might well account for the occurrence of a single species of babesioid in eagles, kestrels, owls, herons, crows, and domestic fowl. The latter quite often live in close association with the other relatively large birds named, apart altogether from the predator–prey relationship. One of us (M.L.) has seen numerous crows (*Corvus splendens*) feeding in poultry runs at Karachi, kestrels settling near fowl houses in the Suez Canal area, and herons mingling with unenclosed fowl on the coasts of Johore and Singapore. Circumstances thus favor the infestation of all these birds with the fowl tick, and *Argas persicus* (Oken), the vector of *Aegyptianella pullorum*, is common in all the localities concerned. According to Hoogstraal (16), this ectoparasite is now found in most parts of the world between 40° N. and 40° S., infesting a variety of domestic birds and also many feral birds nesting near human habitations.

Concentrations of granules of phagocytosed chromatin in some of the white cells on our slide from *Corvus splendens* are morphologically similar to the bodies which Schurenkova (30) interpreted as exoerythrocytic schizonts of *Sogdianella moshkovskii*. This fact lending weight to Mohamed's (23) contention that neither the description nor the figures published by Schurenkova "suggest a parasitic nature for these bodies, and they may represent ordinary granulations or inclusions in these cells", we consider that Schurenkova was dealing with a member of the Babesiidae, not one of the Theileriidae, the family to which she referred her parasite. It is submitted that the avian babesioids recorded by Schurenkova (30), Henry (15), Toumanoff (33), Mohamed (23), and ourselves, although differing from one another in minor respects of size-range and morphology, otherwise have so much in common that they should be regarded as belonging to a single polymorphic species.

By the law of priority the appropriate specific name is that applied by Schurenkova, but the generic designation of *Sogdianella* cannot be sustained on existing data. Poisson (27) regarded *Sogdianella* as a synonym of *Aegyptianella*, but this procedure is unacceptable, as the chief criterion for the latter genus, the formation of large numbers of merozoites from each schizont, is not fulfilled by the former one. It is thought best to follow Wenyon (35) and Toumanoff (33) in using the designation *Babesia* (*sensu lato*), while admitting that this genus stands in need of a critical revision. On the one hand *Babesia*

is a repository for species as different from one another as *B. bigemina* (Smith and Kilborne), which characteristically divides by binary fission and only rarely by schizogony, and the babesioids of the *Nuttallia* type in which fan-shaped and cruciform schizonts producing four merozoites are the rule; while on the other, a much greater range in merozoite number is admitted within the single (and far better understood) genus *Plasmodium*. It may well prove that differences between various members of the family Babesiidae, not forgetting *Aegyptianella*, can best be dealt with at the subgeneric level. Nevertheless, Toumanoff's use of *Nicollia* as a subgeneric designation for the *Babesia* under consideration cannot be endorsed for the reason already given; and there are grounds for contending that *Achromaticus* Dionisi has priority over *Nuttallia* França, which has often been employed as a generic or subgeneric name for Babesiidae producing four merozoites from fan-shaped or cruciform schizonts. Pending clarification of these questions, our parasite is thus identified simply as *Babesia moshkovskii* (Schurenkova, 1938).

Acknowledgment

We are grateful to Dr. C. A. Hoare, F.R.S., for bringing to our attention Dr. A. H. H. Mohamed's unpublished thesis entitled "Protozoal blood parasites of Egyptian birds", a copy of which is in the library of the London School of Hygiene and Tropical Medicine.

References

1. ABDUSSALAM, M. Piroplasmosis of the domestic fowl in northern India. Indian J. Vet. Sci. **15**, 17–21 (1945).
2. BALFOUR, A. A spirillosis and a haematozoal disease of domestic fowls in the Anglo-Egyptian Soudan. Brit. Med. J. (**1**), 744–745 (1907).
3. BALFOUR, A. Spirochaetosis of Sudanese fowls. Wellcome Trop. Research Lab., 4th Rept., 76–107 (1911).
4. BALFOUR, A. Notes on the life-cycle of the Sudan fowl spirochaete. Trans. XVII Intern. Congr. Med., Sect. 21, Pt. 2, 275–278 (1914).
5. BATTELLI, C. Su di un piroplasma della *Naia nigricollis* (*Aegyptianella carpani* n.sp.). Riv. parassitol. **8**, 205–212 (1947).
6. BEDFORD, G. A. H. and COLES, J. D. W. A. The transmission of *Aegyptianella pullorum* Carpano, to fowls by means of ticks belonging to the genus *Argas*. Onderstepoort J. Vet. Sci. Animal Ind. **1**, 15–18 (1933).
7. CARPANO, M. Su di un Piroplasma osservati nei polli in Egitto (*Aegyptianella pullorum*). Nota preventiva. Boll. Serv. Tech. Sci. Min. Agr. Egitto, **86**, 1–12 (1928).
8. CARPANO, M. Sur la piroplasmose des oiseaux domestiques determinée par *Aegyptianella pullorum* (Deuxième note). Ann. parasitol. humaine et comparée, **7**, 365–366 (1929).
9. COLES, J. D. W. A. A new blood parasite of the fowl. Onderstepoort J. Vet. Sci. Animal Ind. **9**, 301–304 (1937).
10. COLES, J. D. W. A. An epizootic in sea birds: a visit to Dassen and Malagas Islands. J. S. African Vet. Med. Assoc. **12**, 23–30 (1941).
11. DELPY, L. P. Description de formes schizogoniques de *Babesia bigemina*. Comparaison avec des formes identiques, decrites par E. Dschunkowsky, 1937, sous le nom *Luhsia bovis* n.sp. Arch. inst. Hessarek, **2**, 43–53 (1946).
12. DSCHUNKOWSKY, E. P. and LUHS, I. Sur l'étude des maladies à protozoaires des oiseaux domestiques en Transcaucasie. (9e Congr. Intern. Med. Vet.) Rev. Gén. Méd. Vét., Toulouse, **15**, 422 (1909).
13. FARID, A. Diseases of animals. Ann. Rept. Vet. Serv. Min. Agr. Egypt (1929–30), 1–6 (1933).
14. FRANCHINI, G. Hematozoaires particuliers d'un oiseau (*Hypoleis hypoleis*). Bull. soc. pathol. exotique, **17**, 884–885 (1924).
15. HENRY, C. H. Presence dans les hématies de poulets d'éléments rappelant les corps de Balfour. Bull. soc. pathol. exotique, **32**, 145–149 (1939).

16. HOOGSTRAAL, H. African Ixodoidea. Vol. 1. Ticks of the Sudan. U.S. Naval Med. Research Rept. NM 005 050.29.07, II + 1–1101 (1956).
17. JAKOWSKA, S. and NIGRELLI, R. F. A taxonomic re-evaluation of *Dactylosoma* Labbé, 1894, a babesioid of cold-blooded vertebrates. J. Protozool. **2**, Suppl. 8 (1955).
18. JAKOWSKA, S. and NIGRELLI, R. F. *Babesiosoma* gen. nov. and other babesioids in erythrocytes of cold-blooded vertebrates. Ann. N.Y. Acad. Sci. **64**, 112–127 (1956).
19. LAIRD, M. The Protozoa of New Zealand intertidal zone fishes. Trans. Roy. Soc. New Zealand, **81**, 79–143 (1953).
20. LAIRD, M. and LARI, FAIYAZ A. Observations on *Plasmodium circumflexum* Kikuth and *P. vaughani* Novy and MacNeal from East Pakistan. J. Parasitol. In press.
21. LINGARD, A. and JENNINGS, E. A preliminary note on a pyroplasmosis found in man and in some of the lower animals. Indian Med. Gaz. **39**, 161–165 (1904).
22. MACNEIL, E. and HINSHAW, W. R. A blood parasite of the turkey. J. Parasitol. **30**, Suppl. 9 (1944).
23. MOHAMED, A. H. H. Protozoal blood parasites of Egyptian birds. Thesis from Dept. Parasitol., London Schl. Hyg. Trop. Med. ,Typescript, VI + 1–424 (1952).
24. NEITZ, W. O. Classification, transmission, and biology of piroplasms of domestic animals. *In* Some protozoan diseases of man and animals: Anaplasmosis, babesiosis, and toxoplasmosis. II. Babesiosis. Ann. N.Y. Acad. Sci. **64**, 56–111 (1956).
25. NEITZ, W. O. and JANSEN, B. C. A discussion on the classification of the Theileridae. Onderstepoort J. Vet. Research, **27**, 7–18 (1956).
26. NEVEU-LEMAIRE, M. Traité de protozoologie médicale et vétérinaire. Vigot Frères, Paris. 1943.
27. POISSON, R. Super-famille des Babesioidea nov. *In* P-P. Grassé's Traité de zoologie. Vol. I. Fasc. II. Masson et Cie., Paris. 1953.
28. PORTER, A. On Anaplasma-like bodies in the blood of vertebrates. Ann. Trop. Med. Parasitol. **9**, 561–568 (1915).
29. ROUSSELOT, R. Parasites du sang de divers animaux de la region de Teheran. Arch. inst. Hessarek, **5**, 62–72 (1947).
30. SCHURENKOVA, A. *Sogdianella moshkovskii* gen. nov. sp. nov.—A parasite belonging to the Piroplasmidea in a raptorial bird—*Gypaëtus barbatus* L. Med. Parazitol. Parazitar. Bolezni, **7**, 932–937 (1938).
31. SERGENT, ED., DONATIEN, A., PARROT, L. and LESTOQUARD, F. Études sur les piroplasmoses bovines. Pasteur Inst., Algiers. 1945.
32. THOMSON, J. G. and HALL, G. N. The occurrence of *Babesia motasi* Wenyon, 1926 in sheep in Northern Nigeria, with a discussion on the classification of the piroplasms. J. Comp. Pathol. Therap. **46**, 218–231 (1933).
33. TOUMANOFF, C. Le parasite sanguin endoglobulaire du héron cendré de l'Indochine (*Ardea cinerea* var. *rectirostris* Gould). *Babesia* (*Nicollia*) *ardeae* nov. sp. Rev. méd. franç. d'extrême-orient, **19**, 491–496 (1940).
34. WASIELEWSKI, T. VON. Zur Kenntnis der Halteridienkrankheit der Vögel. XVII Intern. Congr. Med., Sect. 21, Pt. 1, 245–249 (1914).
35. WENYON, C. M. Protozoology. Baillière, Tindall & Cox, Ltd., London. 1926.

THE DETERMINANTS OF PRODUCTION IN NORTHERN SEAS: A STUDY OF THE BIOLOGY OF THEMISTO LIBELLULA MANDT.

"CALANUS" SERIES NO. 14[1]

M. J. DUNBAR

Abstract

The level of production in the sea is determined not only by the extrinsic factors of the environment, but also by the intrinsic response of the organism to those factors, temperature in particular. In cold seas, the degree of adaptation to low temperature as regards activity, metabolism, growth rate, and the onset of maturity is therefore of great importance. This matter is reviewed and discussed here, and illustrated by a study of the growth rate, breeding cycle, and longevity of a dominant arctic pelagic amphipod, *Themisto libellula* Mandt.

Themisto libellula, in Hudson Bay, Hudson Strait, and the waters of southeast Baffin Island, is shown to have a normal length of life of 2 years, although a 1-year or 15-month life cycle is a possibility in a small proportion of the population. Material from the months of November and December is lacking, but it appears that spawning extends from September through the winter until some time in May or June. If a temperature stimulus is involved in the onset of breeding, it appears to be a fall below, not a rise above, a critical point, since the temperatures during most of the breeding period are below −1.5° C. The spawning in the autumn, however, suggests that temperature is in fact not involved.

The alternating, or two-phase, breeding cycle is confirmed in this species, but the evidence shows that dislocations in the cycle (owing to the possibility of maturation after approximately one year in some individuals) may destroy the supposed reproductive isolation between adjacent year-classes.

Introduction

This paper is a contribution to the study of production in northern waters, production being described in terms of (1) the rate at which organic stuff is made in the seasonal cycle, and (2) the size and density of the resultant standing crop at the height of the seasonal cycle. These, in different trophic levels, depend on a variety of factors, some of them probably still unknown. There is no reason to assume that diatoms, dinoflagellates, zooplankton, fishes, in fact all the poikilothermal elements in the biomass, should be influenced in the same way by the determining environmental factors, or that the production at each level is used to the full by the level above. A large and fast production of phytoplankton, for instance, will make possible a large production of planktonic Crustacea, but it does not follow that the crustacean production will in fact be large, or that it will use the phytoplankton production to the greatest degree possible. There is normally a considerable wastage of plant food by the animals when the former is in great abundance, and a greater or smaller proportion of the plant food will always die unconsumed. The element of wastage was recognized by Volterra (1928 (44)), amongst others, who introduced into his equation for the growth rate of

[1] Manuscript received August 15, 1957.
Contribution from the Department of Zoology, McGill University, and Arctic Unit, Fisheries Research Board of Canada, Montreal, Que.

predator populations a coefficient of food utilization, and Riley (1953 (32)) describes the faecal pellets of zooplankton as being green with discarded and unused plant material following the phytoplankton bloom.

In *terrestrial* polar and mountain environments there is another element which apparently operates so that the maximum possible density of wildlife population is seldom if ever reached, namely the constant or intermittent adverse effects of the climate (Wynne-Edwards, 1952 (47)). In describing the redpoll population in the region of Clyde River, eastern Baffin Island, Wynne-Edwards writes: "Except perhaps among carnivorous predators, competition between individuals for space and nourishment seems commonly to be reduced to a low level among members of the arctic flora and fauna; they live somewhat like weeds, the secret of whose success lies in their ability to exploit transient conditions while they last, in the absence of serious competition. In the arctic the struggle for existence is overwhelmingly against the physical world, now sufficiently benign, now below the threshold for successful reproduction, and now so violent that life is swept away, after which recolonization alone can restore it". This factor, if it applies at all in marine environments, might be expected to take effect only in transition areas between the main zones (arctic, subarctic, boreal), where sudden changes in marine climate might have drastic and possibly differential consequences on parts of the system.

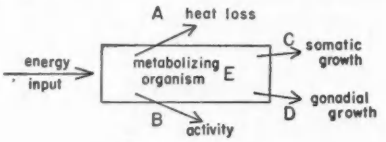


FIG. 1. See text.

Apart from this strictly extrinsic factor, the extent to which the basic (plant) production is used, and therefore the size to which the animal production develops, will depend upon the manner in which the energy obtained from the food itself is used. The energy input fed into an animal (Fig. 1) has to be shared between a number of processes, the relative demands of which, upon the energy capital, are not the same in all environments, or in all animal groups, or at all times of year. It has long been recognized that the activity, for example, of poikilotherms living in very cold circumstances is very much greater than our knowledge of temperature effects upon activity in warmer regions would lead us to expect. There must therefore be regulation, or adaptation, of the determinants of activity which allow the unexpectedly high level in cold regions. Such regulation may be, for all that is at present known about it, either genetically established or a matter of individual ontogenetic adaptation, but it is not difficult to imagine the high probability of the true evolution of such regulation, and its high selective value, since a minimum of activity is required for the business of food-getting and of escaping from predators which are not bound by this temperature condition (homotherms, especially large homotherms).

Regulation in activity with respect to temperature has led zoologists in the past to expect a corresponding, perhaps an enabling, regulation in standard metabolism, and there is evidence that this in fact occurs in many instances. The literature has recently been reviewed by Bullock (1955 (8)). Among the pioneers in the field are Fox (1936 (17*a*), 1939 (17*c*)), Wingfield (1939 (4*b*)); also Fox and Wingfield (1937 (18)) in England, and Thorson (1936 (43*a*), 1950 (43*b*), 1952 (43*c*)) and Spärck (1936 (41)) in Denmark. Thorson, studying the metabolic rates (measured by oxygen consumption) of inshore molluscs in east Greenland, Denmark, and the Persian Gulf, found that the rates were approximately equal in the same species or in closely related species living in the three regions, each being measured at its normal environmental temperature; thus if the arctic individuals were placed in water of normal Danish range of temperature, their metabolic rates were considerably higher than those of the Danish form, and the same, *mutatis mutandis*, for the Danish and Persian Gulf populations. Regulation thus appears to be common, or at least present in a number of species studied.

The work of Fox and Wingfield did not agree with that of Thorson, or of Spärck. Fox (1936 (17*a*)), working on the oxygen consumption of pairs of species (each pair being in the same genus) of echinoderms, annelids, and crustaceans, found that "for seven pairs of species inhabiting respectively English waters and more northern seas (Kristineberg in Sweden), the oxygen consumption of the warmer-water species is greater than that of the colder-water species (measured at the normal temperatures at which each species lives) although the locomotory activity of the former is apparently no greater". Fox suggested "that the similar locomotory activities of the two species require approximately equal amounts of oxygen, but that the non-locomotory oxygen consumption of the warmer-water species is higher than that of the cold-water species". The same results were found for two species of prawns (Fox and Wingfield, 1937 (18)). In this 1937 paper there is a note on the work of Spärck and Thorson, which appeared at the same time. Fox and Wingfield, in commenting on the disagreement between their results and the Danish work, point out that Spärck and Thorson were working on lamellibranchs, which were not used in the English work. Thorson (1952 (43*c*)), and also Berg (1953 (3)), have shown that the animals used in Fox's work were not truly comparable, on account of differences in size and age, and that if these factors are taken into consideration, then metabolic adaptation in the Kristineberg populations was in fact demonstrated.

Wingfield (1939 (46)) showed the danger of drawing too determined conclusions in this matter from comparisons of two different species (which has been the method used by many), by demonstrating that if the polychaete *Pectinaria granulata* from east Greenland is compared with *P. auricoma* from Millport it does not show metabolic regulation, but if compared with *P. koveni*, also from Millport, it does show regulation. Finally Scholander, Flagg, Walters, and Irving (1953 (38)), using this same technique of comparing groups from tropics and arctic which are more or less closely related taxonomically, found that in certain Crustacea there is evidence of metabolic adaptation,

the arctic forms showing somewhat higher metabolism than that which would have been expected simply by extrapolation from the tropical performances.

The species which have been studied are mainly shallow-water benthonic forms; there are as yet no data for the metabolic rates of holoplanktonic species in cold water. Research in this field is now urgently needed and is in fact being planned for the next *Calanus* expedition.

In poikilothermal vertebrates, Fry (1951 (19)) and his collaborators have shown that the speckled trout (*Salvelinus fontinalis*) is capable, individually, of a certain limited metabolic acclimation with respect to temperature, but there is no evidence yet that an established regulation exists in populations living in very cold water. Power (unpublished data), working on metabolic rates of the speckled trout at the northern limit of its range in northern Quebec, has so far found only very low rates which show little or no adaptive regulation. On the other hand Scholander, Flagg, Walters, and Irving (1953 (38)), comparing tropical with arctic fishes in this respect (interspecific and intergeneric comparison) found a considerable adaptation, the arctic species and genera lying in an intermediate position as regards oxygen consumption between the tropical fish and the "expected" or extrapolated position for fishes living at 0° C. Peiss and Field (1950 (29)) compared the oxygen consumption of excised brain and liver tissue of the polar cod (*Boreogadus saida*) with that of the golden orfe (*Idus melanotus*), and found that the Q_{10} values for the cod tissue remained steady over the whole temperature range of 0–25° C., whereas the values for the orfe tissue increased markedly below 10° C. This resulted in oxygen consumption values for the polar cod which were several times as great as in the orfe at low temperatures, clearly advantageous in the adaptation of the polar cod to cold environments. These experiments, however, showed a breakdown in metabolic adaptation in the orfe, the warm-water form, at low temperatures, rather than metabolic regulation in the cod.

There remains the question of growth rates. Both Bullock (1955 (8)) and Dehnel (1955 (9)) have observed that if growth in marine poikilotherms shows adaptation with respect to temperature, as has been shown to be the case for the metabolism in some species, then the high production of certain northern waters would be in large part explained. (It should be emphasized, however, that the high production is in the subarctic waters, not in the coldest, or arctic, regions.) Orton seems to have been one of the first to draw attention to the possibility of adaptation in growth rates. Writing in 1923, he refers to his earlier paper (Orton, 1920 (28*a*)) as drawing attention to the lack of evidence bearing on theories (those of Murray and Loeb, for instance) "to explain (*a*) the abundance of life in polar regions, and (*b*) the occurrence of several generations of species living side by side in polar waters". This latter point, the coexistence of several generations, can now be explained by the prevalence of two-phase or polyphase breeding cycles, such as have been demonstrated for euphausids, the chaetognath *Sagitta elegans* and the amphipod *Themisto libellula* (Ruud, 1932 (35); Dunbar, 1940 (11*a*), 1941 (11*b*), 1946

(11*d*) and this paper). Orton urges "(*a*) that we know nothing about the rate of growth of organisms in polar regions, and (*b*) that the kind of metabolism of animals in polar regions and in deep-sea situations is not necessarily the same as that in temperate and tropical regions".

Orton (1923 (28*b*)) found that sea urchins (identified as *Echinus miliaris*) observed in west Spitsbergen showed a rate of growth of the same order as is found in related forms in Plymouth waters in the English Channel. Again, this is the comparison of different species, not of populations of the same species. Thorson (1936 (43*a*)), studying lamellibranchs in east Greenland inshore waters, found slow growth rates, and generalizing from his work on the lamellibranchs, concluded "that the bottom invertebrates in the northeast Greenland seas will on the whole have a slow growth, a long life, and a late maturity which means that the production must be extremely small". He adds that this generalization is provisional, and that investigation of more species is necessary. Later (43*b*), finding that larval growth in arctic waters appears to be fast, he treats adaptive regulation of both metabolism and growth as a generally accepted phenomenon, and on those grounds dismisses Wimpenny's (1941 (45)) paper on "Organic polarity" as being out of date, since Wimpenny assumed that life cycles were shorter in tropical than in polar waters. He makes the observation that the *larvae* of benthonic invertebrates, northern as well as tropical, "seem on the average to spend a similar time (about three weeks) in the plankton", at the temperatures to which they are normally exposed, but he adds that in arctic regions abnormally low temperatures can have an adverse effect, and that "wastage caused by temperature, like that due to starvation, seems mainly to be an indirect one: low temperatures postpone growth and metamorphosis, and give the enemies a longer time to feed on the larvae".

Dehnel (1955 (9)) compared the growth rates, expressed as instantaneous relative rates, of the larvae of gastropod molluscs in southeastern Alaskan and southern Californian waters, and found that the rates for the northern populations were from two to nine times greater than for the southern populations of the same species over given ranges of temperatures. This comparison was intraspecific, populations of the same species being used. In spite of the fact that species with yolky telolecithal egg masses were chosen, to minimize the importance of food availability on growth, it is still possible that factors other than temperature were responsible for the fast growth of the Alaskan embryos, as Dehnel himself points out. Two other comments on these very interesting results are relevant here: (1) the surface temperatures of the southeastern Alaskan waters are not low when compared to arctic or even subarctic regions (the annual range at Sitka is from about 4° C. to 15° C.); and (2) the growth measured was embryonic and larval. It has been pointed out by several workers, including Thorson (1936 (43*a*), 1950 (43*b*)) that there are special problems for the colder-water benthonic forms, in that it is essential, for survival, that metamorphosis be accomplished within a certain short time after hatching, the time being determined by the length of the high production period in the phytoplankton, which is usually short in cold

regions, owing to the great stability of the surface waters in summer. Most arctic benthonic molluscs have solved this problem by eliminating the pelagic larval stage.

A high growth rate in embryonic development was also found by Fox (1938 (17*b*)) in sea urchins, those of British waters developing faster than those of the Mediterranean, at given temperatures.

The growth rates of larval and embryonic stages, however, are only a very small part of the problem. From the point of view of the student of production in the northern seas, the important thing is the time taken to reach maturity and breeding. Moreover, there is no reason to assume that what is found in the molluscs and in the echinoderms is true of all groups; and finally, the benthonic and littoral forms may behave quite differently in this respect from the holoplanktonic. Dehnel (1955 (9)) writes: "Generally, the assumption is made that species of marine poikilotherms which inhabit the colder waters of the higher latitudes grow more slowly, grow to a larger size, and have a greater longevity than individuals of the same or closely related species from warmer waters". In fact, this is much more than an assumption, as Dehnel indicates later in his paper. There is a great deal of evidence in the literature for the slowing down of growth rates in cold water as compared with warm, not only in the comparison of different species within one genus but also in the comparison of different populations within one species. Thus Schneider (1891 (37)) concludes that several of the common gammarid amphipods of the Tromsö coast have a 2-year life cycle, some even longer, and comments that increase in life-time, and time taken to reach maturity, with increasing geographic latitude is a well-known phenomenon in insects. In the Lepidoptera, for instance, many species have a 1-year life cycle in Germany and a 2-year cycle in Norway. Schneider mentions also the tiger-moth, *Arctia caja*, which lives 1 year in the Oslo area but which requires 2 years to reach maturity at Tromsö.

In the chaetognath *Sagitta elegans*, Russell (1932 (34)) recorded four, possibly five broods, produced in 1 year at Plymouth, each brood being parental to the next. In the same species in Baffin Island waters, Dunbar (1941 (11*b*)) found the normal life cycle to be probably as long as 2 years before spawning. Bogorov (1940 (5)) found a 2-year life cycle for both *S. elegans* and *Eukrohnia hamata* in the Barents Sea. In the Euphausiidae, Macdonald (1928 (23)) recorded a breeding cycle in *Thysanoessa raschii*, in the Clyde Sea area, which appeared to involve a 1-year life cycle. Einarsson (1945 (13)) also found a 1-year cycle for the same species in southern Icelandic waters, but concluded that in northern Iceland and in west Greenland *T. raschii* took 2 years to reach maturity. Bogorov (1940 (5)) recorded a 2-year cycle for *Thysanoessa inermis* and *T. longicaudata* in the Barents Sea. In the deep-water shrimp *Pandalus borealis*, Hjort and Ruud (1938 (22)) concluded that maturation (to maleness) occurred in the second summer of life in Norwegian waters; in the same species Rasmussen (1942 (30)) described a 3-year maturity age in Spitsbergen.

The copepod *Calanus finmarchicus* has been exhaustively studied. The researches have recently been reviewed and consolidated by Marshall and Orr (1955 (25)), who write (p. 64): "As is natural, the lower the temperature the slower is the rate of development, and the later does the first spawning occur". In the Clyde Sea area, where the temperature at the time it is homothermic in March is about 7° C., *Calanus finmarchicus* spawns at least three times during the period between mid-February and late July. Thus the life span in spring is about 2 months. The winter stock may live up to 7 months. In east Greenland (Digby, 1954 (10*b*)), where the winter temperature is about −1.5° C. for a considerable period, the same species spawns only once a year, in midsummer, and the individual normal life span is therefore 1 year. There is evidence that a proportion of the population may survive a second winter. In Ungava Bay, Fontaine (1955 (16)) found 1-year life cycles in *Calanus finmarchicus* and *Pseudocalanus minutus*. Other planktonic copepods follow the same pattern. Digby (1954 (10*b*)) comments that in view of this, copepods in the arctic cannot be considered to be particularly well adapted to living in cold seas. There is, however, some likelihood that the availability of food may be an important factor, besides temperature, in determining the growth rate.

An exception to this apparent rule of slower growth in colder water was reported by Moore (1934 (26)), who found that the common barnacle, *Balanus balanoides*, has a faster growth at Herda, in Norway, than at Port Erin in the Irish Sea, where the growth was in turn faster than at St. Malo in France.

In vertebrate poikilotherms the evidence is confusing, as indeed it is also in the invertebrates. The arctic char (*Salvelinus alpinus*) in Frobisher Bay, Baffin Island, takes 12 years to reach maturity in the female (Grainger, 1953 (20*a*)). The Atlantic cod (*Gadus callarias*) of Port Burwell, Ungava Bay, grows considerably more slowly than the cod of west Greenland, Newfoundland, or Nova Scotia (Dunbar, unpublished). In these instances there is no evidence that the food supply is involved. In the Amphibia, however, the work of Moore (1939 (27*a*), 1942 (27*b*), etc.) has shown that the embryonic development of species of *Rana* is faster, the colder the environment, the northern *Rana sylvatica* developing at about twice the rate of the southern *Rana catesbeiana*. Differences in the type of life cycle are probably at the root of the ultimate (as opposed to the proximate) causes for the differences between groups in this respect, which, as has been suggested above is the case between the benthonic invertebrates and the holoplanktonic forms.

Material and Methods

The hyperiid amphipods have been given scant attention biologically. The tropical and temperate species are difficult taxonomically and appear to be of secondary importance in the economy of the seas. The cold-water species, however, *T. libellula* in particular, are abundant and often dominant. As a group the hyperiids are well suited to studies of the present sort, being large, easily caught, holoplanktonic or holopelagic, with direct development.

Themisto libellula is a dominant species of the plankton of the waters of Hudson Strait, north and east Baffin Island, and Hudson Bay. Its ecological position farther north is not yet known (it is not abundant in Foxe Basin), but it appears to be arctic rather than subarctic, being not common in the west Greenland waters except in their northern part. It extends, however, into the subarctic on the Canadian side, decreasing in abundance southward along the Labrador Current. In the subarctic it can be relied upon as an indicator of arctic water. It has been recorded from all arctic seas explored, except that information from the Arctic Ocean itself is lacking. The most southerly records are the Strait of Belle Isle (Bousfield, 1951 (6)), off Cape Farewell and the southeast coast of Iceland (Stephensen, 1923 (42)), the Norwegian, Barents, and Murmansk seas, Kara Sea, Bering and Okhotsk seas (Behning, 1939 (2)).

The species figures prominently in the food of the arctic char (Grainger, 1953 (20)), and especially of the ringed seal (Dunbar, 1941 (11*c*)). It forms the most important link in the food chain between the copepods and other smaller planktonic forms on the one hand, and the vertebrates on the other, and in fact it takes the place, in cold water, of the euphausids in this respect.

The material used in the present paper was collected on the *Calanus* Expeditions of 1947 to 1955, the details of the collection being shown in Tables I and II. Open plankton nets were used, and most of the *Themisto* were taken in horizontal hauls. The nets were the 1- and 2-meter stramin nets, a half-meter No. 6 net and No. 5 net of the same size, and 1-meter No. 0 and No. 00 nets. Depths of hauling are approximate only, being calculated from the wire angle and the length of wire. An error of about 10% can be expected in the depth calculations. Material was also obtained from stomachs of the ringed seal (*Phoca hispida*) in the waters of southwest Baffin Island (Cape Dorset) and from Igloolik in Foxe Basin; and from stomachs of arctic char at the head of Frobisher Bay.

TABLE I

COLLECTION DATA FOR THE *Themisto libellula* MATERIAL (IN THE "NET" COLUMN, NUMBERS REFER TO THE BOLTING SILK GRADE, EXCEPT THAT "STR. 1" MEANS "1-METER STRAMIN; "STR. 2", "2-METER STRAMIN")

Year	Date	Station	Depth, m.		Net	Time, E.S.T.	Duration, min.	Numbers	
			Station	Haul				Taken	Measured
1947	24/6	1	18–31	5	6	1420	25	7	7
	29/6	3	2	2	Str. 1	1100	30	2	2
	29/6	3	28	4–5	6	1104	25	9	9
	29/6	3	28	5–7	0	1104	25	42	41
	13/7	13	46–55	6	0	1000	30	80	80
	17/7	18	84	17–21	0	1240	30	50	50
	10/8	33	15	11–13	Str. 1	0810	30	2	2
	10/8	33	15	11	0	0810	30	9	9
	11/8	37	3–4	0	6	1040	30	1	1
	11/8	37	3–4	0	Str. 1	1048	30	1	1
	17/8	41	240	27–35	6	1300	60	1	1
	17/8	41	240	200	0	1300	60	15	15
	17/8	41	240	54	Str. 1	1300	60	Several hundreds	2
	19/8	44	80	70	0	1330	40	1	1
	19/8	44	80	70	Str. 1	1334	36	18	18
1949	26/6	101	18	6–8	Str. 1	1045	30	8	8
	26/6	101	18	1	6	1055	30	3	3
	6/7	103	145–275	90	00	—	20	20	20
	6/7	103	145–275	200	Str. 1		45	14	14
	14/7	103	145–275	105	Str. 1	0930	45	44	44

Year	Date	Station	Depth, m. Station	Depth, m. Haul	Net	Time, E.S.T.	Duration, min.	Numbers Taken	Numbers Measured
1949	7/8	103	145–275	5–10	00	1120	12	9	9
	10/8	123	15	1–7	5	1515	35	1	1
	10/8	123	15	5–10	00	1645	22	3	3
	21/8	125	18	0	Str. 1	—	90	1	1
	23/8	126	70–90	2–10	Str. 1	1057	35	37	37
	24/8	128	185	27	00	1445	70	43	43
	24/8	128	185	137–162	Str. 1	1455	70	25	25
	25/8	129	–	16–19	Str. 1	1340	60	3	3
	25/8	129	–	3	00	1340	60	3	3
	25/8	129	–	13–15	6	1345	50	4	4
1950	28/6	201C	80–100	1	00	0935	55	11	11
	28/6	201C	80–100	20	Str. 1	0955	20	13	13
	15/7	206	55–90	3	Str. 1	1020	45	60	60
	20/7	209	183	75	Str. 1	1305	25	78	78
	3/8	222	80–90	8	5	1800	20	42	42
1951	4/7	303	120	–	Str. 1	1715	30	64	64
	28/7	309	165	75–87	Str. 1	2000	30	43	43
	14/8	326	550–640	225	Str. 1	1015	60	139	139
	21/8	329	183	150	Str. 1	1153	50	47	47
	26/8	335	320	50	Str. 1	1715	15	55	55
	26/8	335	320	92	Str. 1	1715	15	11	11
	26/8	335	320	155–215	Str. 1	1855	30	90	90
	26/8	335	320	0	0	2016	15	24	24
	26/8	335	320	50	Str. 1	2016	15	77	77
	26/8	335	320	92	Str. 1	2016	15	115	115
	26/8	335	320	0	0	2312	15	192	192
	26/8	335	320	50	Str. 1	2312	15	72	72
	26/8	335	320	92	Str. 1	2312	15	67	67
	26/8	335	320	154	Str. 1	2352	30	86	86
	27/8	335	320	0	0	0209	15	207	207
	27/8	335	320	50	Str. 1	0209	15	48	48
	27/8	335	320	92	Str. 1	0209	15	46	46
	27/8	335	320	20–25	Str. 1	0250	15	105	105
	27/8	335	320	250	Str. 1	0407	15	30	30
	27/8	335	320	0	0	0505	15	7	7
	27/8	335	320	50	Str. 1	0505	15	10	10
	27/8	335	320	92	Str. 1	0505	15	3	3
1952	29/7	407	228	115	Str. 1	1010	15	12	12
	29/7	407	228	10	5	1122	15	12	12
	2/8	411	83–91	0	18	0920	12	48	48
	5/8	414	275–365	105	Str. 1	1810	35	216	216
	6/8	416	275–285	115	Str. 1	1535	20	37	37
	6/8	416	275–285	175	Str. 1	1620	45	512	512
1953	13/7	502	22–27	20	0	1615	10	21	21
	21/7	503	65–90	35	0	1520	25	86	86
	21/7	503	65–90	17	Str. 2	1700	15	53	53
	31/7	514	20	9	0	1703	30	15	15
	4/8	520	137	87	0	1250	30	55	55
	4/8	520	137	40–52	Str. 2	1615	15	Several hundred	121
	9/8	527	355–415	62	0	1110	20	43	43
	11/8	531	183	62	0	1710	25	16	16
	17/8	535	140–155	7	5	1320	15	66	54
	4/9	549	75	7	5	1210	20	63	63
	4/9	549	75	46	Str. 2	1345	15	43	43
	8/9	512	21	14	Str. 2	1440	25	32	32
	8/9	512	21	15	0	1545	20	49	49
1954	24/7	603	90–110	40	Str. 2	1600	30	6	6
	27/7	603	90–110	26	Str. 2	1721	15	Several hundred	76
	5/8	606	99	26	Str. 2	0000	30	87	87
	5/8	606	99	43–65	Str. 2	0140	15	265	265
	12/8	613	189	78–115	Str. 2	0955	30	175	175

TABLE II

COLLECTION DATA FOR *Themisto libellula* MATERIAL FROM STOMACHS OF RINGED SEAL (*Phoca hispida*)

Year	Date	Location	Number measured
1953	23/8	Cape Dorset	230
1954	22/3	Cape Dorset	315
1954	13–19/7	Cape Dorset	184
1955	27/10	Igloolik	112
1956	31/1	Igloolik	3
1956	27/2	Igloolik	17
1956	28/2	Igloolik	10

The 1947 and 1949 material comes entirely from Ungava Bay; the 1948 material consists of a collection of *Themisto* taken from the stomachs of arctic char at the head of Frobisher Bay (see map Fig. 2). The 1951 collection is in the main from Frobisher Bay, 1952 from Cumberland Sound. (For the identification of the stations in Table I, see the station lists already published (Dunbar and Grainger, 1952 (12); Grainger, 1954 (20*b*); Grainger and Dunbar, 1956 (21).) The 1953 and 1954 material is from northern Hudson Bay and the western end of Hudson Strait. Collections from ringed seal stomachs in 1953 and 1954 are from the region of Cape Dorset, southwest Baffin Island (August 1953, March and July 1954), and the material from October 1955 to March 1956 is taken from ringed seals off Igloolik, in the north of Foxe Basin.

Measurements were made with a low-power binocular microscope, the specimens being placed on a thin glass slide over a half-millimeter scale. The over-all lengths, from the front of the head to the tip of the longest uropods, were read to the nearest quarter-millimeter and then grouped on integer centers, all individuals from $x \cdot 5$ to $(x+1) \cdot 25$ being grouped under $(x+1)$ mm. The length frequencies of the samples are presented in the histograms (Figs. 3 to 5 and 11 to 13).

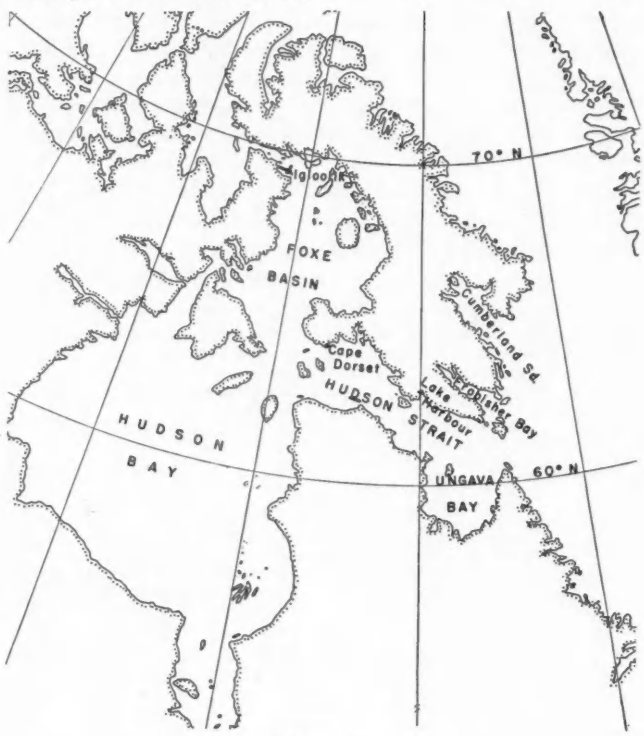


FIG. 2.

The Environment

The detailed hydrography of the area will be published in a paper to appear shortly in this series. Hudson Bay, Foxe Basin, and the western part of Hudson Strait are arctic areas in which the influence of Atlantic water is minimal if indeed it is present at all. Frobisher Bay, although probably containing a small proportion of west Greenland water, with Atlantic admixture, in the summer, is nevertheless predominantly arctic. Ungava Bay, on the other hand, shows considerable Atlantic influence, both hydrographically and in its fauna. It is an unstable region with very large tidal intervals and is slightly warmer below 25 meters than the rest of the region considered here. July temperatures in Hudson Bay and Hudson Strait (western part), below 25 meters, are normally below $-1°$ C., whereas they range from $-1.5°$ C. up to about $+1.5°$ C. in Ungava Bay. In August, the 50-meter water may be up to 2° warmer in Ungava Bay than in the rest of the region, including Frobisher Bay. The 100-meter water in August in Ungava Bay is similar in temperature range to the water at the same depth in Froblisher Bay, between 0° and $-1°$ C., and about 0.5° warmer than the Hudson Bay level, where the 100-meter water is below $-1.0°$. Hudson Bay, being highly stratified in summer owing to the influx of fresh water from the large land area it drains, is distinctive in having the surface water warmed up to the level of about 10° C. As far as the present paper is concerned, however, these exceptional temperatures are of no significance, since *Themisto* is apparently inhibited by them from invading the surface layers.

Growth Rate and Breeding Cycle

In an earlier paper on *Themisto* in Baffin Island waters (Dunbar, 1946 (11*d*)) it was shown that the size frequencies of the samples were strongly bimodal, the two size-groups being statistically quite separate and therefore representing two separate broods. The same bimodal distribution is demonstrated in the present material, shown in Figs. 4, 5, 11, and 12. The division between the two groups occurs, in the summer period of collecting, at about 20 mm.

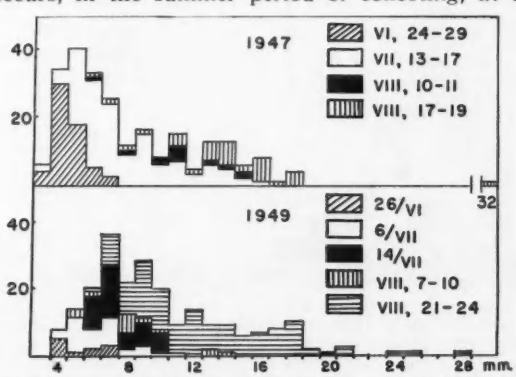


FIG. 3. Size distribution in *Themisto libellula* taken at different times during the summer seasons of 1947 and 1949, Ungava Bay.

In the separation of the broods for statistical treatment, the lowest point in the distribution has been taken, and this point is seen (Fig. 12) to coincide with the point at which, in the ascending series of length, adolescent individuals begin to appear, in contrast to the juvenile population to the left of the low point.

Figs. 3, 4, and 5 show the material from 1947 and 1949 (Ungava Bay), 1951 (Ungava Bay, Lake Harbour, and (predominantly) Frobisher Bay), and 1953 (northern Hudson Bay, and the western end of Hudson Strait), arranged according to date of collecting, to demonstrate change in size during the sum-

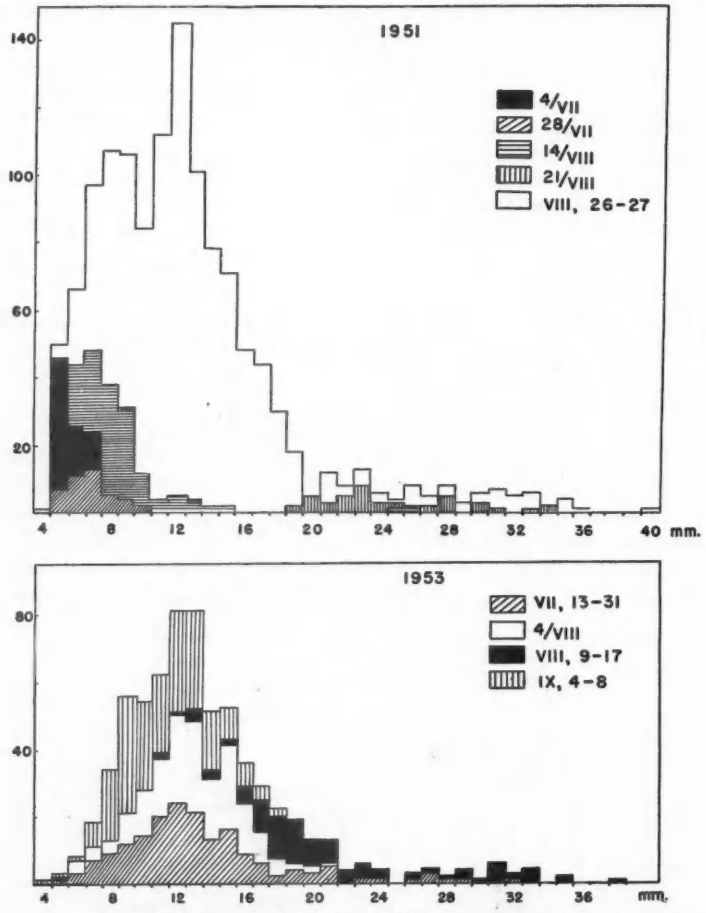
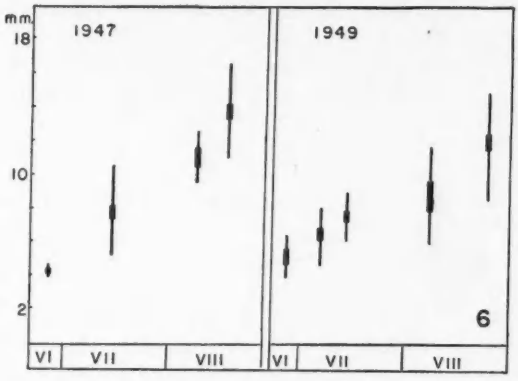


FIG. 4. Size distribution in *Themisto libellula* taken on 5 days in 1951, in Ungava Bay (4/VII), Lake Harbour (28/VII), and Frobisher Bay.

FIG. 5. Size distribution in *Themisto libellula* taken at different times during the summer season of 1953, in northern Hudson Bay and the western entrance of Hudson Strait.

mer season. In Figs. 6, 7, and 8 the increase in body-size in the populations is shown graphically on either side of the mean by lines representing one standard deviation (thin lines) and one standard error (thick lines), the total length of each line being therefore two standard deviations.



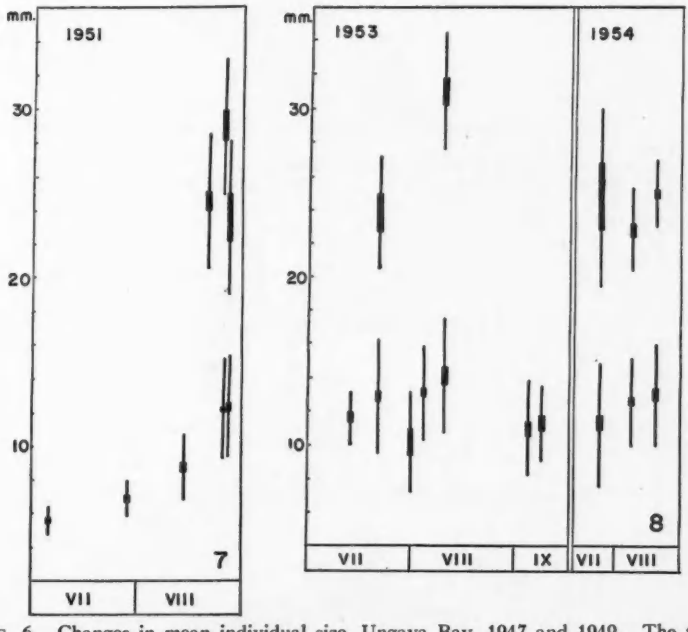
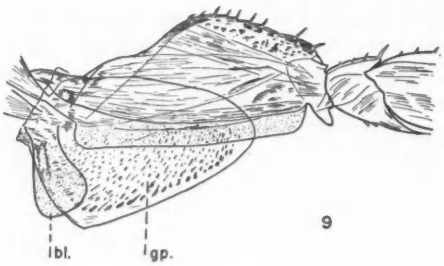


FIG. 6. Changes in mean individual size, Ungava Bay, 1947 and 1949. The total length of the vertical lines represents two standard deviations, that of the broad section two standard errors. Months on the horizontal axis.

FIG. 7. Changes in mean individual size, Ungava Bay (early July), Lake Harbour (late July), and Frobisher Bay (August). (For details see Fig. 6.)

FIG. 8. Changes in mean individual size, 1953 (northern Hudson Bay and west entrance of Hudson Strait), and 1954 (Hudson Bay). (For details see Fig. 6.)



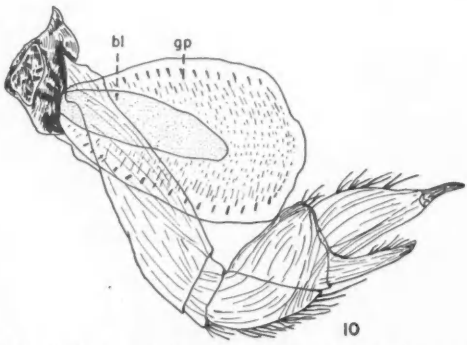


FIG. 9. Part of the fifth peraeopod of a female in the stage referred to as "adolescent" in the text.

Abbreviations: bl., brood lamella; gp., gill plate.

FIG. 10. Second leg of a female in the "maturing" stage, in which the brood lamella (bl.) is increasing in size.

Abbreviation: gp., gill plate.

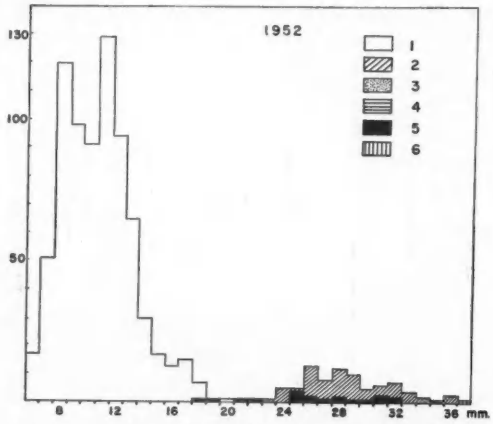


FIG. 11. Size distribution of *Themisto libellula* from Cumberland Sound, 1952, showing maturity stages.

The key refers to Figs. 11, 12, and 13; (1) immature; (2) "adolescent" females; (3) "maturing" females; (4) mature females; (5) adolescent males; (6) mature males. Vertical axis, numbers of individuals; horizontal axis, length in mm.

The material from 1952 (Cumberland Sound), 1954 (Northern Hudson Bay), and from stomachs of the ringed seal (Cape Dorset area, 1953, 1954, and Igloolik, 1955) has been divided into maturity stages, and the results shown in Figs. 11, 12, and 13. The stages are based on the development of the brood lamellae, or oostegites, in the female, and on the growth of the antennae in the male. Mature specimens were not taken in the net hauls, and the seal stomach material was too far digested to allow the use of the ovaries and testes. The delimitation of the stages is as follows:

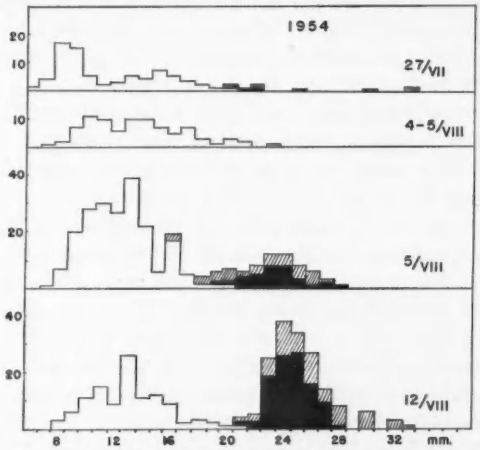


FIG. 12. Size distribution of *Themisto libellula* from northern Hudson Bay, 1954, taken on various dates, showing maturity stages. (Details as in Fig. 11.)

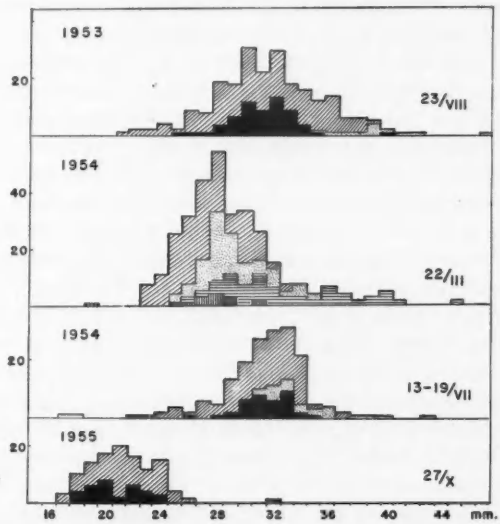


FIG. 13. Size distribution of *Themisto libellula*, with maturity stages, from ringed seal stomachs. 1953, 1954: Cape Dorset; 1955: Igloolik.

Immature: Antennae very short, no sign of development of oostegites.

Adolescent female: Oostegites present, but rudimentary, as in Fig. 9. Ovaries small, usually less than 5 mm. in length, cells very small.

Maturing female: Oostegites increasing in size from the "adolescent" condition (Fig. 10). Ovaries larger, with cells about 0.2 mm. in diameter or larger.

Mature female: Oostegites fully developed, almost twice the length of the gill plates. Specimens often with eggs or young in the brood pouch.

Adolescent male: Antennae increasing in length (Dunbar, 1946 (11*d*), Fig. 5).

Mature male: Antennae fully developed, the first antennae reaching to the hind margin of the first pleonal segment, the second antennae reaching beyond the hind margin of the last pleonal segment.

It has been demonstrated (Dunbar, 1946 (11*d*)) that in *Themisto libellula* there is an alternating or two-phase breeding cycle, such that two generations, or broods, exist at the same time, the two broods being apparently isolated from each other so far as reproduction is concerned, unless the cycle goes out of phase or unless a few individuals breed twice. During the breeding period itself, three broods are present, the breeding group, the adolescent group, and the very young group which is being produced at the time. Breeding cycles of this sort have also been shown to be the rule in certain other cold-water pelagic forms. It was not clear, however, from the earlier work, whether there were breeding periods in the fall and in the early part of the year (March–May), in which case the length of life would be 1 year, or whether there was only one breeding period, in which case the length of life would of necessity be 2 years at least.

Figs. 6, 7, and 8 are not growth curves; they show the manner in which the size-frequencies of *Themisto* in the plankton samples change as the summer advances. It will be seen that in 1947 and 1949 (Ungava Bay) and in 1951 (predominantly Frobisher Bay) there is an orderly progression of advancing average size in the youngest, or juvenile, group. This increase is less clear in the 1954 material, and in 1953, also from Hudson Bay, the slope is definitely reversed by the reduction in average size of the population in September. Fig. 5, dealing with the same 1953 population, shows that this effect in September is caused by the entry into the sample of numbers of very small individuals, down to 4 mm. in length, recently freed from the parental brood pouch. A similar arrival of very small specimens, this time in late August, is also shown in the Frobisher Bay collection of 1951 (Fig. 4), but here the average size of the juvenile population is not reduced compared to samples from earlier in the season owing to the presence of large numbers of larger juveniles, up to 19 mm. in length. There is no such late appearance of small individuals within the collecting season (June–August) in Ungava Bay (Fig. 3).

Another important difference between the Ungava Bay samples on the one hand and those from Frobisher Bay, Hudson Bay, and western Hudson Strait on the other is the almost complete absence of the larger (adolescent) group in Ungava Bay. In the net plankton, only one individual taken in 1947 belonged to the adolescent group, and only nine in 1949. The ringed seal

(*Phoca hispida*) feeds habitually on the larger individuals of the *Themisto* population (Dunbar, 1941 (11*c*)) yet of 36 ringed seals taken in the 1947–1950 seasons, only 10 had *Themisto* in the stomach, and those 10 had a total of only 36 specimens between them. Of these 36 specimens, only one half were over 20 mm. in length, and the largest was only 25 mm. This is in strong contrast to the seal stomachs taken at Lake Harbour, Frobisher Bay (Dunbar, 1941 (11*c*)), and in the Cape Dorset area (McLaren, in press), which were frequently found to be full of *Themisto libellula*, often to the exclusion of other food organisms. A swarm of adolescent individuals was observed and sampled in 1948 at the western end of Ikeresak, or McLelan Strait, which separates the island upon which Port Burwell is situated from the mainland of Labrador, but in that highly turbulent region it is impossible to determine where the swarm came from or whether it represented a permanent population. The evidence is in favor of the conclusion that *Themisto* does not breed in Ungava Bay, that the juvenile population is brought into the bay from the northwest, in the Hudson Strait outgoing current, and that it is swept out of the bay again before it reaches adolescence; or that it does not survive within the bay for some other reason. There are in fact certain hydrographic differences between Ungava Bay and the rest of the area examined here which are referred to above and which might be adequate to explain the matter.

Not one mature specimen, male or female, was taken in the plankton nets in any season. The maturity stages of the net catches of 1952 and 1954 are shown in Figs. 11 and 12, and they are typical of all years. Nor were there any mature specimens taken in seal stomachs in summer. As shown in Fig. 13, however, seal stomachs taken in March 1954, in the Dorset area, contained mature males and females, and mature females occurred in stomachs taken in February 1956 at Igloolik. Stomachs examined in August 1953 and July 1954 contained females in the maturing condition. The absence of maturing females from the net hauls is unexplained. It is improbable that individuals of this size can escape a 2-meter stramin net hauled at 3 knots, especially at night; it may be, however, that the maturing females maintain a lower level of vertical distribution than the others, although the net was lowered close to the bottom on several occasions and hauled obliquely, still without taking the maturing individuals.

Mature females, with and without eggs and young in brood pouches, were taken only in late January, February, and March. The young are released at a size of 2 to 3 mm. In the present series no collecting was done earlier in the winter than the end of January, and summer field work was carried out only between late June and early October. However, since the juvenile samples taken in June and early July show a fairly wide range of body length, and include individuals of only 4 mm., it may safely be assumed that the breeding period extends over a considerable period and that new broods are still being shed in the first half of June. The absence of spent individuals in spring and summer is evidence that each individual breeds once only (see below). We do not know how long the young are retained in the brood pouch.

One mature male was taken in the stomach of an arctic char in Frobisher Bay in August 1948 (precise date in August not given). This was an individual 31 mm. in length with antennae fully developed (first antenna 17.5 mm., second antenna 20.5 mm.), with sexual papillae developed, and with the testes silvery, rounded, and swollen and the seminal duct darkly pigmented. Another male 22 mm. in length had a second antenna 11 mm. long, first antennae missing. The females in the char stomach material from the same month were not mature, but some were in the "maturing" category, up to 32.5 mm. in length.

The presence of the mature male in August, the maturing females in seal and char stomachs in July and August, the one mature female taken in a seal stomach in October 1955 at Igloolik (Fig. 13), and the recruitment of very small juveniles into the stock in late August 1951 (Fig. 4) and early September 1953 (Fig. 5)—all indicate that breeding begins in the late summer and early fall. It is possible that this autumn breeding period is less important than the breeding period in the early part of the year. However, the lack of material in November, December, and January makes it impossible to rule out the possibility of a sustained breeding period throughout the winter, from September perhaps to June, and indeed for the greater part of the year.

Themisto libellula does not appear to be abundant in Foxe Basin, but there is a little material from ringed seal stomachs taken at Igloolik in January and February 1956, which is helpful. From January 31 there are three measurable specimens, 28, 30, and 31 mm. long, all with fully developed oostegites, and parts of 13 other specimens of similar size. From February 27 there are 20 specimens, one male of 27.5 mm. with the antennae broken but clearly well developed, and 19 females of which three are not measurable. The remaining 16 range from 20.0 to 35 mm. in length, and they all have fully developed oostegites, some with eggs and early young. The small size of the smaller of these specimens (20–21.5 mm.) suggests that at least some of the juveniles of each year may come into breeding condition the following winter. From February 28 there is a collection of 11 specimens of which nine are mature females ranging from 26 to 36 mm., one is a mature female of similar size with the head missing, and one is a juvenile of 11 mm. Clearly spawning has started by the month of February if not before, and although it is improbable that many of the juveniles of the year breed in the following winter, some apparently do, as shown by the small specimens taken on February 27, 1956. Most of the breeders each winter, however, must be the adolescents of the previous summer; indeed, the strong bimodality of the size-frequencies in summer makes it impossible that many of the juveniles should breed the winter after they are produced. This means that the life cycle of the individual is between 18 months and 2 years, but that a life cycle of 1 year is not impossible. This argument is illustrated in Fig. 14, and rests on the assumption that each individual breeds only once. The fact that a 1-year life cycle is possible means that the reproductive isolation of the two coexisting year-classes is not complete.

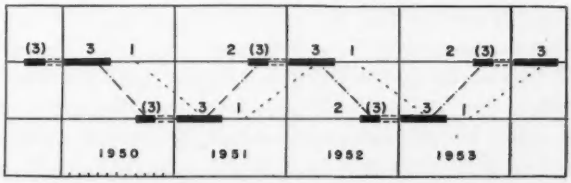


FIG. 14. Schematic diagram representing the probable breeding cycle of *Themisto libellula*. The two horizontal lines represent the time-continua of the two populations which exist together in the same space, between which reproductive contact is probably quite small, or absent. The heavy black portions represent the breeding periods; (1) immature group; (2) adolescent group; (3) mature breeding group. The possible extension of breeding throughout the long winter is indicated by the horizontal dotted lines. The diagonal lines represent the cases in which the alternating cycle may go out of phase either by the holdover of breeding until the fall, or by the precocious maturation of small individuals (Group 1).

Discussion

1. The question of whether *Themisto libellula* breeds more than once in its life is obviously highly important in reaching an understanding of the breeding cycle. The evidence on this point is not entirely conclusive, and in the past there has been some difference of opinion on this point as regards the amphipods as a group. Schneider (1891 (37)) quotes three authorities on the question and gives some observations of his own. Thus Siebold and Stannius (1854 (40)) describe the brood lamellae or oostegites of the female amphipod as developing rapidly at the approach of the breeding season and later disappearing or becoming atrophied. Bate (1856 (1)) considered that there was no evidence for the loss of the brood lamellae. Bruzelius (1859 (7)) was willing to allow the possibility of decrease in size of brood lamellae after breeding, but did not believe that they disappeared completely. Schneider himself pointed out that all three authors appeared to have assumed that amphipod individuals breed more than once, but that there was no evidence of that. He suggested that the absence of spent specimens, and of large females without oostegites, could most simply be explained by supposing that breeding only once per lifetime was normal, as it is in insects.

Blegvad (1921 (4)) records "*Gammarus locusta*" (probably *G. zaddachi*) as breeding seven or eight times per female in Danish waters, within a short time in one breeding season, and finds that the stock produced after the month of May can breed in two seasons; that is, after breeding once in late summer they may survive the winter and breed again the following spring. In Segerstråle's (1950 (39)) paper on the amphipods of Finnish waters, on the other hand, there is no indication that *Gammarus zaddachi* (or its "*salinus*" form) breeds more than once; the cold-water *Gammarus oceanicus*, similarly, appears to breed only once per individual.

In the present study it has been concluded that the *Themisto libellula* individual breeds once and dies. There is no evidence whatsoever to the contrary. No spent specimens of either sex were taken, except in March at the height of the breeding season.

2. The stimulus for the final ripening of the gonads and the onset of the breeding, if it has anything to do with temperature at all, appears to involve a fall rather than a rise in temperature. Orton (1920 (28)) suggested that "Most animals under normal conditions begin to breed either at a definite temperature, which is a physiological constant for the species, or at a definite temperature change, namely at either the maximum or the minimum temperature of the locality", and added that many continued to breed so long as the temperature remained above or below a certain figure, critical for each species, and that a given species might be a winter breeder in warmer situations and a summer breeder in colder areas, a situation which appears to be illustrated in the invertebrate fauna of Norwegian waters, where the northern elements breed in the winter and the southern elements in the summer (Ekman, 1953 (14)). Orton took the argument further: "It follows that in those parts of the sea where temperature conditions are constant or nearly constant, and where biological conditions do not vary much, marine animals will breed continuously". He suggested that this condition might be found in the arctic. In the abyssal depths of the sea, where there may well be no seasonal variation in temperature, breeding all year may well be the case, as is stated, apparently on rather slim evidence, by Reibisch (1927 (31)), although even in the abyssal region there must be a seasonal variation of the food supply, which comes from the surface layers. On the other hand, year-round breeding is known to occur in species where habitats certainly do show considerable seasonal temperature variation, as in *Gammaris zaddachi zaddachi* on the coast of Finland (Segerstråle, 1950 (39)). In arctic waters, however, Orton's prognostication has not been realized. All arctic and subarctic forms so far studied have shown well-marked breeding periods, often extending over only a short part of the year. Indeed, year-round breeding appears to be far more common in *tropical* waters, not in the arctic.

There is to begin with a significant variation in the annual temperature cycle at the depths in which the animals of the arctic and subarctic continental shelf live. Thus in Godthaab Fjord, a decidedly subarctic region as opposed to arctic, the annual range of temperature at 75 meters is of the order of 1° C. to 2.75° C. (Dunbar, 1946 (11*e*)). Surface temperatures of course show a wider range, but they are not ecologically significant in the present argument. In Scoresby Sound, east Greenland, Digby (1953 (10*a*)) recorded, at 50 meters, −0.95° C. at the beginning of October, −1.78° in January, and −1.47° at the end of July. From November to May inclusive the temperature was not above −1.72° C. at 50 meters depth. In the waters of northern Baffin Island, Ellis (1956 (15)) measured, again at 50 meters, temperatures of −0.90° C. in September and October, −1.00° in November, −1.05° in December, −1.70° to −1.75° in April and again in June, −1.65° in late June, and −1.45° in July. A similar cycle was recorded at Igloolik by the *Calanus* wintering expedition of 1955–56: from November through the winter and spring to June, the temperatures at 50 meters were below −1.70° C.,

in July rose to −1.63°, in August to −0.07°, and in September the temperature measured was −0.24°. Hudson Bay conditions are essentially similar. In the area between Southampton Island, Coats Island, and Mansel Island temperatures at 50 meters in July and August range from −1.50° to about 0.05°, and in the months of April, May, and June they are below −1.70° down to −1.80° C. Winter temperatures in Frobisher Bay and Ungava Bay are not known, but the upper 50–75 meters in Ungava Bay in summer are both warmer and less stable, temperatures as high as 2.16° C. having been recorded.

The coldest time of the year, therefore, in the depths at which *Themisto libellula* lives, in arctic regions, is from November to June inclusive, when the temperature is close to its "floor" level of about −1.70°. From July to early October temperatures are about 2° higher. Therefore, if temperature controls the onset of breeding, and since *Themisto* breeds in the coldest part of the year, it is indicated that the stimulus is a fall below certain threshold value and not a rise above it. However, the fact that breeding begins, apparently, in September, close to the warmest part of the year in the water, suggests that the temperature threshold is not the overriding or efficient stimulus, but that some other factor such as light is involved. It is possible that the autumn breeders should be considered as "hold-overs" from the end of the previous winter, breeding late for one reason or another, and that they are not in the normal physiological condition. More information is needed on the winter biology of the species.

It is well known that arctic species which extend south into the boreal region tend to breed during the winter, but it is not yet established that winter breeding in the arctic itself is a common habit. As a winter breeder of egregiously arctic affinities, *Themisto libellula* contrasts with another important member of the arctic pelagic fauna, *Sagitta elegans arctica* Aurivillius, which breeds in the summer (Dunbar, 1941 (11*b*)), and with the planktonic copepods (Fontaine, 1955 (16); Digby, 1954 (10*b*)). Samter and Weltner (1904 (36)) reported of *Mysis relicta* in Europe that it produced eggs only in cold water, below 7° C., and that the chief production was in the winter, at temperatures between 0° and 3° C.

The appearance of large numbers of very small amphipods released from the parental brood pouches in the dead of winter raises the question of what they eat. The density of the visible or sedimentable phytoplankton during the winter is extremely low, and the first sign of phytoplankton development at Igloolik during 1955–56 did not appear until the first half of May (Grainger, unpublished). Two possibilities remain: either the young feed almost entirely on detritus, perhaps close to the bottom or on it, or else there must be a continuing production of microplankton which, as Rodhe (1955 (33)) suggests for subarctic lakes in Sweden, becomes heterotrophic in the winter, using dissolved organic matter. Indeed, dissolved organic matter, in the form of leptopelic detritus, may play an important part in the direct diet of the young amphipods.

Acknowledgment

The author is indebted to Dr. E. H. Grainger, Arctic Unit, Fisheries Research Board of Canada, for a considerable proportion of the measurements of the *Themisto* material, and for the plotting and organization of some of them; also for the temperature data from the winter observations at Igloolik, Northwest Territories, 1955–56.

References

1. BATE, C. S. On the British Edriophthalma. Part I; 'The Amphipoda. Rept. Brit. Assoc. Advance. Sci. 1855. 18–62, 1856.
2. BEHNING, A. L. Die Amphipoda-Hyperiidea der den Fernen Osten der USSR umgrenzenden Meere. Internat. Rev. ges. Hydrobiol. Hydrog. **38**, 353–367 (1939).
3. BERG, K. The problem of respiratory acclimatization. Hydrobiologica, **5** (4), 331–350 (1953).
4. BLEGVAD, H. Om nogle danske Gammaridens og Mysidens Biologi. Beretn. Danske Biol. Sta. **28**, 1–120 (1921).
5. BOGOROV, B. G. On the biology of the Euphausiidae and Chaetognatha in the Barents Sea. Bull. Soc. Naturalistes Moscou, N.S. **49** (2), 3–18 (1940).
6. BOUSFIELD, E. L. Pelagic amphipoda of the Belle Isle Strait region. J. Fisheries Research Board Can. **8** (3), 134–163 (1951).
7. BRUZELIUS, R. M. Bidrag til kännedomen om Skandinaviens Amphipoda Gammaridea. Kgl. Svenska Vetenskapsakad. Handl., Ny Följd, **3**, 1–104 (1859).
8 BULLOCK, T. H. Compensation for temperature in the metabolism and activity of poikilotherms. Biol. Revs. **30** (3), 311–342 (1955).
9. DEHNEL, P. A. Rates of growth of gastropods as a function of latitude. Physiol. Zool. **28** (2), 115–144 (1955).
10*a*. DIGBY, P. S. B. Plankton production in Scoresby Sound, East Greenland. J. Animal Ecol. **22** (2), 289–322 (1953).
10*b*. DIGBY, P. S. B. The biology of the marine plankton copepods of Scoresby Sound, East Greenland. J. Animal Ecol. **23** (2), 298–338 (1954).
11*a*. DUNBAR, M. J. On the size distribution and breeding cycles of four marine planktonic animals from the arctic. J. Animal Ecol. **9** (2), 215–226 (1940).
11*b*. DUNBAR, M. J. The breeding cycle in *Sagitta elegans arctica* Aurivillius. Can. J. Research, D, **19** (9), 258–266 (1941).
11*c*. DUNBAR, M. J. On the food of seals in the Canadian Eastern Arctic. Can. J. Research, D, **19**, 150–155 (1941).
11*d*. DUNBAR, M. J. On *Themisto libellula* in Baffin Island coastal waters. J. Fisheries Research Board Can. **6** (6), 419–334 (1946).
11*e*. DUNBAR, M. J. The state of the West Greenland current up to 1944. J. Fisheries Research Board Can. **6** (7), 460–471 (1946).
12. DUNBAR, M. J. and GRAINGER, E. H. Station list of the "Calanus" expeditions, 1947–1950. J. Fisheries Research Board Can. **9** (2), 65–82 (1952).
13. EINARSSON, H. Euphausiacea: I. Northern Atlantic species. Carlsberg Foundation, Dana Report No. 27, 1–185 (1945).
14. EKMAN, S. Zoogeography of the Sea. Sidgwick & Jackson, London. 1953.
15. ELLIS, D. V. Some temperature and salinity records from the Canadian Arctic during 1954 and 1955. J. Fisheries Research Board Can. **13** (4), 591–598 (1956).
16. FONTAINE, M. The planktonic copepods (Calanoida, Cyclopoida, Monstrilloida) of Ungava, with special reference to the biology of *Pseudocalanus minutus* and *Calanus finmarchicus*. J. Fisheries Research Board Can. **12** (6), 858–898 (1955).
17*a*. FOX, H. M. The activity and metabolism of poikilothermal animals in different latitudes. I. Proc. Zool. Soc. London, 1936, 945–955 (1936).
17*b*. FOX, H. M. The activity and metabolism of poikilothermal animals in different latitudes. III. Proc. Zool. Soc. London, 1938, Ser. A, 501–505 (1938).
17*c*. FOX, H. M. The activity and metabolism of poikilothermal animals in different latitudes. V. Proc. Zool. Soc. London, 1939–40, Ser. A, 141–156 (1939).
18. FOX, H. M. and WINGFIELD, C. A. The activity and metabolism of poikilothermal animals in different latitudes. II. Proc. Zool. Soc. London, 1937, Ser. A, 275–282 (1937).
19. FRY, F. E. J. Some environmental relations of the speckled trout (*Salvelinus fontinalis*). Proc. N. E. Atlantic Fisheries Conference, May 1951, 1–29 (1951).
20*a*. GRAINGER, E. H. On the age, growth, migration, reproductive potential and feeding habits of the arctic char (*Salvelinus alpinus*) of Frobisher Bay, Baffin Island. J. Fisheries Research Board Can. **10** (6), 326–370 (1953).

20*b*. GRAINGER, E. H. Station list of the "Calanus" Expeditions, 1951–52, together with Frobisher Bay stations, 1948, 1950 and 1951, and Resolution Island stations, 1950. J. Fisheries Research Board Can. **11** (1), 98–105 (1954).
21. GRAINGER, E. H. and DUNBAR, M. J. Station list of the "Calanus" Expeditions, 1953–54. J. Fisheries Research Board Can. **13** (1), 41–45 (1956).
22. HJORT, J. and RUUD, J. T. Deep sea prawn fisheries and their difficulties. Hvalrådets Skrifter, Nr. **17**, 1–144 (1938).
23. MACDONALD, R. The life history of *Thysanoessa raschii*. J. Marine Biol. Assoc. United Kingdom, **15** (1), 55–66 (1928).
24. MCLAREN, I. A. The biology of the ringed seal (*Phoca hispida* Schreber) in the eastern Canadian arctic. J. Fisheries Research Board Can. (In press).
25. MARSHALL, S. M. and ORR, A. P. The biology of a marine copepod. Oliver & Boyd, Ltd., Edinburgh. 1955.
26. MOORE, H. B. The biology of *Balanus balanoides*. I. Growth rate and its relation to size, season and tidal level. J. Marine Biol. Assoc. United Kingdom, **19**, 851–868 (1934).
27*a*. MOORE, J. A. Temperature tolerance and rate of development in the eggs of Amphibia. Ecology, **20**, 459–478 (1939).
27*b*. MOORE, J. A. The role of temperature in speciation in frogs. Biol. Symposia, **6**, 187–213 (1942).
28*a*. ORTON, J. H. Sea-temperature, breeding and distibution in marine animals. J. Marine Biol. Assoc. United Kingdom, **12**, 339–366 (1920).
28*b*. ORTON, J. H. Some experiments on rate of growth in a polar region (Spitzbergen) and in England. Nature, **111**, 146–148 (1923).
29. PEISS, C. N. and FIELD, J. The respiratory metabolism of excised tissues of warm- and cold-adapted fishes. Biol. Bull. **99**, 213–224 (1950).
30. RASMUSSEN, B. Om dypvannsreken ved Spitsbergen. *Rept. Norweg. Fisheries and Marine Invest.* **7** (4), 1–44 (1942).
31. REIBISCH, J. Amphipoda. Handbuch der Zoologie (Kükenthal und Krumbach), **3** (1), 767–808 (1927).
32. RILEY, G. A. Theory of growth and competition in natural populations. J. Fisheries Research Board Can. **10** (5), 211–223 (1953).
33. RODHE, W. Can plankton production proceed during winter darkness in subarctic lakes? Proc. Intern. Assoc. Theoret. and Appl. Limnology, **12**, 117–122 (1955).
34. RUSSELL, F. S. On the biology of Sagitta. The breeding and growth of *Sagitta elegans* Verrill in the Plymouth area, 1930–31. J. Marine Biol. Assoc. United Kingdom, **18**, 131–145 (1932).
35. RUUD, J. T. On the biology of the southern Euphausiidae. Hvalrådets Skrifter, Nr. **2**, 1–105 (1932).
36. SAMTER, M. and WELTNER, W. Biologische Eigentümlichkeiten der *Mysis relicta*, *Pallasiella quadrispinosa* und *Pontoporeia affinis* erklärt aus ihrer eiszeitlichen Entstehung. Zool. Anz. **27**, 676–694 (1904).
37. SCHNEIDER, J. S. Forplantningstiden og Livsvarigheden hos Amphipoderne. Tromsö Museums Aarshefter, **14**, 59–74 (1891).
38. SCHOLANDER, P. F., FLAGG, W., WALTERS, V. and IRVING, L. Climatic adaptation n arctic and tropical poikilotherms. Physiol. Zool. **26**, (1), 67–92 (1953).
39. SEC ERSTRÅLE, S. G. The amphipods on the coasts of Finland — some facts and problems. Soc. Sci. Fennica Commentationes Biol. **10** (14), 1–28 (1950).
40. SIEBOLD, K. VON and STANNIUS, H. Comp. Anat. Vol. I. *Translated by* W. I. Burnett. Gould & Lincoln, Boston. 1854.
41. SPÄRCK, R. On the relation between metabolism and temperature in some marine lamellibranchs and its ecological and zoogeographical importance. Kgl. Danske Videnskab. Selskab., Biol. Medd. **13**, 1–27 (1936).
42. STEPHENSEN, K. Crustacea malacostraca 5. Amphipoda (1). Danish *Ingolf* Expedition **3** (8), 1–100 (1923).
43*a*. THORSON, G. The larval development, growth and metabolism of arctic marine bottom invertebrates. Medd. Grønland, **100**, 1–71 (1936).
43*b*. THORSON, G. Reproductive and larval ecology of marine bottom invertebrates. Biol. Revs. **25**, 1–45 (1950).
43*c*. THORSON, G. Zur jetzigen Lage der marinen Bodentier-Okologie. Verhandl. deut. Zool. Ges in Wilhelmshaven, 1951, **34**, 276–327 (1952).
44. VOLTERRA, V. Variations and fluctuations of the number of individuals in animal species living together. J. Conseil, **3**, 1–51 (1928).
45. WIMPENNY, R. S. Organic polarity; some ecological and physiological aspects. Quart. Rev. Biol. **16** (4), 389–425 (1941).
46. WINGFIELD, C. A. The activity and metabolism of poikilothermal animals in different latitudes. IV. Proc. Zool. Soc. London, 1939–40, Ser. A, 103–108 (1939).
47. WYNNE-EDWARDS, V. C. Zoology of the Baird Expedition (1950). I. The birds observed in central and south-east Baffin Island. Auk, **69**, 353–391 (1952).

CONTENTS OF VOLUME 35

Anderson, R. C. Observations on the life cycles of *Diplotriaenoides translucidus* Anderson and members of the genus *Diplotriaena*, 15.

Taxonomic studies on the genera *Aproctella* Cram, 1931 and *Carinema* Pereira and Vaz, 1933 with a proposal for a new genus *Pseudaproctella* n. gen., 25.

Backs, R. H. See Friend, W. G., 535.

Bandy, P. J., Kitts, W. D., Wood, A. J., and **Cowan, I. McT.** The effect of age and the plane of nutrition on the blood chemistry of the Columbian black-tailed deer (*Odocoileus hemionus columbianus*). B. Blood glucose, non-protein nitrogen, total plasma protein, plasma albumin, globulin, and fibrinogen, 283.

Battle, H. I. See Hall, J. C., 593; Shaw, B. L., 325.

Belle, E. A. Helminth parasites of reptiles, birds, and mammals in Egypt. IV. Four new species of oxyurid parasites from reptiles, 163.

Berkeley, C. See Berkeley, E., 573.

Berkeley, E. and **Berkeley, C.** On some pelagic Polychaeta from the northeast Pacific north of latitude 40° N. and east of longitude 175° W., 573.

Bernard, R. See Huot, L., 513.

Bose, R. J. See Kitts, W. D., 449.

Cass, L. M. See Friend, W. G., 535.

Connell, R. and **Corner, A. H.** *Polymorphus paradoxus* sp. nov. (Acanthocephala) parasitizing beavers and muskrats in Alberta, Canada, 525.

Coppel, H. C. and **Smith, B. C.** Studies on dipterous parasites of the spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). V. *Omotoma fumiferanae* (Tot.) (Diptera: Tachinidae), 581.

Corner, A. H. See Connell, R., 525.

Cowan, I. McT. See Bandy, P. J., 283; Kitts, W. D., 449.

Dionne, L. A. and **Spicer, P. B.** A squash method for somatic chromosomes of aphids, 711.

Downe, A. E. R. Precipitin test studies on rate of digestion of blood meals in black flies (Diptera: Simuliidae), 459.

Dunbar, M. J. The determinants of production in northern seas: A study of the biology of *Themisto libellula* Mandt., 797.

Edmunds, G. F., Jr. *Metretopus borealis* (Eaton) in Canada (Ephemeroptera: Ametropodidae), 161.

Fairlie, T. W. See Rothfels, K. H., 221.

Fallis, A. M. and **Wood, D. M.** Biting midges (Diptera: Ceratopogonidae) as intermediate hosts for *Haemoproteus* of ducks, 425.

Freeman, R. S. Life cycle and morphology of *Paruterina rauschi* n. sp. and *P. candelabraria* (Goeze, 1782) (Cestoda) from owls, and significance of plerocercoids in the order Cyclophyllidea, 349.

Friend, W. G., Backs, R. H., and **Cass, L. M.** Studies on amino acid requirements of larvae of the onion maggot, *Hylemya antiqua* (Mg.), under aseptic conditions, 535.

Gibbs, H. C. Helminth parasites of reptiles, birds, and mammals in Egypt. III. *Cyathospirura seurati* sp. nov. from *Fennecus zerda*, 201.

The taxonomic status of *Rictularia affinis* Jägerskiöld, 1909, *Rictularia cahirensis* Jägerskiöld, 1909, and *Rictularia splendida* Hall, 1913, 405.

Greenbank, D. O. The role of climate and dispersal in the initiation of outbreaks of the spruce budworm in New Brunswick. II. The role of dispersal, 385.

Hall, J. C. and **Battle, H. I.** Respiratory movements and endurance of frogs at low pressures, 593.

Hanson, H. C., Levine, N. D., and **Ivens, V.** Coccidia (Protozoa: Eimeriidae) of North American wild geese and swans, 715.

Harris, E. Radiophosphorus metabolism in zooplankton and microorganisms, 769.

Hart, J. S. Seasonal changes in CO_2 sensitivity and blood circulation in certain fresh-water fishes, 195.

See also Irving, L., 497.

Harvey, G. T. The occurrence and nature of diapause-free development in the spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae), 549.

Hoar, W. S. See Irvine, D. G., 691.

Houston, A. H. Responses of juvenile chum, pink, and coho salmon to sharp sea-water gradients, 371.

Huot, L., Bernard, R., and **Lemonde, A.** Aspects quantitatifs des besoins en minéraux des larves de *Tribolium confusum* Duval. I. Pourcentage optimum d'un mélange salin, 513.

Irvine, D. G., Newman, K., and **Hoar, W. S.** Effects of dietary phospholipid and cholesterol on the temperature resistance of goldfish, 691.

Irving, L. and **Hart, J. S.** The metabolism and insulation of seals as bare-skinned mammals in cold water, 497.
Ivens, V. See Hanson, H. C., 715.
Khan, M. A. The effect of dietetic and glycemic factors in the attractiveness of white rats to mosquitoes, 189.
Sphaerularia bombi Duf. (Nematoda: Allantonematidae) infesting bumblebees and *Sphaerularia hastata* sp. nov. infesting bark beetles in Canada, 519.
Sphaerularia ungulacauda sp. nov. (Nematoda: Allantonematidae) from the Douglas fir beetle, *Dendroctonus pseudotsugae* Hopk., with key to *Sphaerularia* species (emended), 635.
Kingscote, A. A. See Wu, L.Y., 207.
Kitts, W. D. See Bandy, P. J., 283.
Kitts, W. D., Bose, R. J., Wood, A. J., and **Cowan, I. McT.** Preliminary observations on the digestive enzyme system of the beaver (*Castor canadensis*), 449.
Laird, M. and **Lari, F. A.** The avian blood parasite *Babesia moshkovskii* (Schurenkova, 1938), with a record from *Corvus splendens* Vieillot in Pakistan, 783.
Lari, F. A. See Laird, M., 783.
Lemonde, A. See Huot, L., 513.
Levine, N. D. See Hanson, H. C., 715.
Lubinsky, G. Studies on the evolution of the Ophryoscolecidae (Ciliata: Oligotricha). I. A new species of *Entodinium* with "*caudatum*," "*loboso-spinosum*," and "*dubardi*" forms, and some evolutionary trends in the genus *Entodinium*, 111.
Studies on the evolution of the Ophryoscolecidae (Ciliata: Oligotricha). II. On the origin of the higher Ophryoscolecidae, 135.
Studies on the evolution of the Ophryoscolecidae (Ciliata: Oligotricha). III. Phylogeny of the Ophryoscolecidae based on their comparative morphology, 141.
Buxtonella sulcata (Ciliata: Holotricha), a parasite of *Bos indicus* and *Bubalus bubalus* in the Western Punjab, 545.
Note on the phylogenetic significance of predatory habits in the Ophryoscolecidae (Ciliata: Oligotricha), 579.
List of helminths from Alberta rodents, 623.
McCraw, B. M. Studies on the anatomy of *Lymnaea humilis* Say, 751.
Mahon, J. *Ophryocotyle brasiliensis* sp. nov. (Davaineidae) from *Hoploxypterus cayanus* (Lath.), 279.
Deltokeras synallaxis sp. nov. (Dilepididae) from *Synallaxis rutilans* Temm., 441.
Mann, P. M. and **Stinson, R. H.** Activity of the short-tailed shrew, 171.
Mawson, P. M. Filariid nematodes from Canadian birds, 213.
Miller, C. A. A technique for estimating the fecundity of natural populations of the spruce budworm, 1.
Montreuil, P. L. J. and **Ronald, K.** A preliminary note on the nematode parasites of seals in the Gulf of the St. Lawrence, 495.
Mulvey, R. H. Taxonomic value of the cone top and the underbridge in the cyst-forming nematodes *Heterodera schachtii*, *H. schachtii* var. *trifolii*, and *H. avenae* (Nematoda: Heteroderidae), 421.
Myers, B. J. Nematode parasites of seals in the Eastern Canadian Arctic, 291.
Ascaroid parasites of harp seals (*Phoca grœnlandica* Erxleben) from the Magdalen Islands, Quebec, 291.
Newman, K. See Irvine, D. G., 691.
Ogilvie, J. M. G. and **Warren, A. A.** The occurrence of trimethylamine oxide in *Fundulus heteroclitus*, 735.
Pelluet, D. The effect of low temperature on the ascorbic acid content of the ovotestis of the slug, *Arion subfuscus* (Draparnaud), 609.
Plummer, P. J. G. See Sweatman, G. K., 93.
Rempel, J. G. The embryology of the black widow spider, *Latrodectus mactans* (Fabr.), 35.
Riordan, D. F. Effects of a high temperature on the fertility of *Dahlbominus fuscipennis* (Zett.) (Hymenoptera: Chalcidoidea), 603.
Robertson, J. G. Paper chromatography in insect taxonomy, 411.
Somatic metaphase chromosomes in geographic isolates of the carrot rust fly, *Chamaepsila rosae* (F.) (Diptera: Psilidae), 453.
Changes in resistance to DDT in *Macrocentrus ancylivorus* Rohw. (Hymenoptera: Braconidae), 629.
Ronald, K. The metazoan parasites of the Heterosomata of the Gulf of St. Lawrence. I. *Echinorhynchus laurentianus* sp. nov. (Acanthocephala: Echinorhynchidae), 437.
The metazoan parasites of the Heterosomata of the Gulf of St. Lawrence. II. *Entobdella curvunca* sp. nov. (Trematoda: Capsalidae), 747.
See also Montreuil, P. L. J., 495.
Rothfels, K. H. and **Fairlie, T. W.** The non-random distribution of inversion breaks in the midge *Tendipes decorus*, 221.

Sanwal, K. C. The morphology of the nematode *Radopholus gracilis* (de Man, 1880) Hirschmann, 1955, parasitic in roots of wild rice, *Zizania aquatica* L., 75.
Chambersiellidae n. fam. (Nematoda) with emended diagnosis of the genus *Chambersiella* Cobb, 1920, description of *C. bakeri* n. sp., and discussion of taxonomic position, 615.
Shaw, B. L. and **Battle, H. I.** The gross and microscopic anatomy of the digestive tract of the oyster *Crassostrea virginica* (Gmelin), 325.
Smith, B. C. See Coppel, H. C., 581.
Spicer, P. B. See Dionne, L. A., 711.
Squires, H. J. Decapod crustacea of the *Calanus* Expeditions in Ungava Bay, 1947 to 1950. *Calanus* Series No. 11, 463.
Stinson, R. H. See Mann, P. M., 171.
Strachan, A. A. Eye worms of the family Thelaziidae from Brazilian birds, 179.
Sweatman, G. K. Life history, non-specificity, and revision of the genus *Chorioptes*, a parasitic mite of herbivores, 641.
Sweatman, G. K. and **Plummer, P. J. G.** The biology and pathology of the tapeworm *Taenia hydatigena* in domestic and wild hosts, 93.
Virkki, N. Structure of the testis follicle in relation to evolution in the Scarabaeidae (Coleoptera), 265.
Warren, A. A. See Ogilvie, J. M. G., 735.
Wellington, W. G. Individual differences as a factor in population dynamics: The development of a problem, 293.
Wood, A. J. See Bandy, P. J., 283; Kitts, W. D., 449.
Wood, D. M. See Fallis, A. M., 425.
Wu, L. Y. and **Kingscote, A. A.** Studies on *Trichinella spiralis*. II. Times of final molt, spermatozoa formation, ovulation, and insemination, 207.

CORRECTIONS

Volume 34, page 590. In line 13, "A cirrus was not seen in 17 of 26 flukes examined." should read "A cirrus was seen in 17 of 26 flukes examined."

Volume 35, pages 75–92. In Figs. 1 and 2 the oesphageal gland lobe should overlap the intestine ventrally and the caudal alae in Fig. 2 should point towards the bottom of the page. The error has been caused by the wrong juxtaposition of three components of the original drawing, which, being too large to be drawn in one field, had to be constructed in several small components. In Fig. 8 there should be only four incisures as described in the text.

Notes to Contributors

Manuscripts

(i) ***General***

Manuscripts, in English or French, should be typewritten, double spaced, on paper 8½ × 11 in. **The original and one copy are to be submitted.** Tables and captions for the figures should be placed at the end of the manuscript. Every sheet of the manuscript should be numbered.

Style, arrangement, spelling, and abbreviations should conform to the usage of this journal. Names of all simple compounds, rather than their formulas, should be used in the text. Greek letters or unusual signs should be written plainly or explained by marginal notes. Superscripts and subscripts must be legible and carefully placed.

Manuscripts and illustrations should be carefully checked before they are submitted. Authors will be charged for unnecessary deviations from the usual format and for changes made in the proof that are considered excessive or unnecessary.

(ii) ***Abstract***

An abstract of not more than about 200 words, indicating the scope of the work and the principal findings, is required, except in Notes.

(iii) ***References***

References should be listed **alphabetically by authors' names,** numbered, and typed after the text. The form of the citations should be that used in this journal; in references to papers in periodicals, titles should be given and inclusive page numbers are required. The names of periodicals should be abbreviated in the form given in the most recent *List of Periodicals Abstracted by Chemical Abstracts.* All citations should be checked with the original articles, and each one referred to in the text by the key number.

(iv) ***Tables***

Tables should be numbered in roman numerals and each table referred to in the text. Titles should always be given but should be brief; column headings should be brief and descriptive matter in the tables confined to a minimum. Vertical rules should be used only when they are essential. Numerous small tables should be avoided.

Illustrations

(i) ***General***

All figures (including each figure of the plates) should be numbered consecutively from 1 up, in arabic numerals, and each figure should be referred to in the text. The author's name, title of the paper, and figure number should be written in the lower left-hand corner of the sheets on which the illustrations appear. Captions should not be written on the illustrations (see Manuscripts (i)).

(ii) ***Line drawings***

Drawings should be carefully made with India ink on white drawing paper, blue tracing linen, or co-ordinate paper ruled in blue only; any co-ordinate lines that are to appear in the reproduction should be ruled in black ink. Paper ruled in green, yellow, or red should not be used unless it is desired to have all the co-ordinate lines show. All lines should be of sufficient thickness to reproduce well. Decimal points, periods, and stippled dots should be solid black circles large enough to be reduced if necessary. Letters and numerals should be neatly made, preferably with a stencil (**do NOT use typewriting**), and be of such size that the smallest lettering will be not less than 1 mm. high when reproduced in a cut 3 in. wide.

Many drawings are made too large; originals should not be more than 2 or 3 times the size of the desired reproduction. In large drawings or groups of drawings the ratio of height to width should conform to that of a journal page but the height should be adjusted to make allowance for the caption.

The original drawings and one set of clear copies (e.g. small photographs) are to be submitted.

(iii) ***Photographs***

Prints should be made on glossy paper, with strong contrasts. They should be trimmed so that essential features only are shown and mounted carefully, with rubber cement, on white cardboard with no space or only a **very** small space (less than 1 mm.) between them. In mounting, full use of the space available should be made (to reduce the number of cuts required) and the ratio of height to width should correspond to that of a journal page (4¾ × 7½ in.); however, allowance must be made for the captions. Photographs or groups of photographs should not be more than 2 or 3 times the size of the desired reproduction.

Photographs are to be submitted in duplicate; if they are to be reproduced in groups one set should be mounted, the duplicate set unmounted.

Reprints

A total of 50 reprints of each paper, without covers, are supplied free. Additional reprints, with or without covers, may be purchased.

Charges for reprints are based on the number of printed pages, which may be calculated approximately by multiplying by 0.6 the number of manuscript pages (double-spaced typewritten sheets, 8½ × 11 in.) and including the space occupied by illustrations. An additional charge is made for illustrations that appear as coated inserts. The cost per page is given on the reprint requisition which accompanies the galley.

Any reprints required in addition to those requested on the author's reprint requisition form must be ordered officially as soon as the paper has been accepted for publication.

Contents

PAGE

Sphaerularia ungulacauda sp. nov. (Nematoda: Allantonematidae) from the Douglas Fir Beetle, *Dendroctonus pseudotsugae* Hopk., with Key to *Sphaerularia* Species (Emended)—*M. A. Khan* - - - - - - - 635

Life History, Non-specificity, and Revision of the Genus *Chorioptes*, a Parasitic Mite of Herbivores—*Gordon K. Sweatman* - - - - - - 641

Effects of Dietary Phospholipid and Cholesterol on the Temperature Resistance of Goldfish—*D. G. Irvine, Katherine Newman, and W. S. Hoar* - 691

A Squash Method for Somatic Chromosomes of Aphids—*Leo A. Dionne and P. B. Spicer* - - - - - - - - - - - - - - - 711

Coccidia (Protozoa: Eimeriidae) of North American Wild Geese and Swans—*Harold C. Hanson, Norman D. Levine, and Virginia Ivens* - - - - - - 715

The Occurrence of Trimethylamine Oxide in *Fundulus heteroclitus*—*Jane M. G. Ogilvie and A. A. Warren* - - - - - - - - - - 735

The Metazoan Parasites of the Heterosomata of the Gulf of St. Lawrence. II. *Entobdella curvunca* sp. nov. (Trematoda: Capsalidae)—*Keith Ronald* - 747

Studies on the Anatomy of *Lymnaea humilis* Say—*Bruce M. McCraw* - - 751

Radiophosphorus Metabolism in Zooplankton and Microorganisms—*Eugene Harris* - - - - - - - - - - - - - - - 769

The Avian Blood Parasite *Babesia moshkovskii* (Schurenkova, 1938), with a Record from *Corvus splendens* (Vieillot) in Pakistan — *Marshall Laird and Faiyaz A. Lari* - - - - - - - - - - - - - - 783

The Determinants of Production in Northern Seas: A Study of the Biology of *Themisto libellula* Mandt. —*M. J. Dunbar* - - - - - - - 797

Contents of Volume 35 - - - - - - - - - - - - - - 821